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# OXIDATION PROCESSES OCCURRING IN THE SYSTEM PLASMA (SERUM)—POTASSIUM FERRICYANIDE.

By G. LITARCZEK.

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IN a paper<sup>(1)</sup> dealing with oxygen dissociation curves in anæmic rabbits attention will be drawn to a phenomenon which is more marked with sera of anæmic animals than of normal, using Barcroft's differential manometer and the ferricyanide method. It is stated there that constant readings for the oxygen total capacity cannot be secured in advanced cases of anæmia with a manifest lipæmia, and therefore these values are incorrect as well as those directly connected with the oxygen total capacity, the *K* of Hill's formula bearing therefore a corresponding error. Evidence was obtained that an oxygen consumption accompanies the phenomenon, to which the above error was due. This process has nothing to do with the well-known phenomenon of cell respiration described by Morawitz and Rohmer<sup>(2)</sup> in 1908, but seems to be identical with that already described by these authors, as well as by Douglas<sup>(3)</sup> in 1910 using the Haldane ferricyanide method, and occurs as well in whole blood as in plasma or serum.

Since the introduction of potassium ferricyanide in the technique of blood gas analysis by Haldane in 1900<sup>(4)</sup>, many difficulties have been observed in gas analysis using the same type of apparatus and ferricyanide.

Besides the above-mentioned authors, Van Slyke and Stadie<sup>(5)</sup> observed an  $O_2$  consumption in an alkaline medium. As a further sign of unsatisfactory results obtained with the ferricyanide method, and that the phenomenon has been observed by others, is the fact that some authors, Krogh, Lundsgaard<sup>(6)</sup>, preferred to estimate the total oxygen capacity from the hæmoglobin values.

Although the phenomenon seems to have been definitely observed no systematic work on it has been found. On the occasion of a short communication of my first results made to the Hæmoglobin Committee of the Medical Research Council on March 5th, 1927, I had the opportunity of discussing them with Dr C. G. Douglas<sup>(7)</sup>, who discovered the phenomenon and who is also working at it. Douglas pointed out the

importance of the proteins. Two communications by Parsons(8), (9), were made on May 14th to the Physiological Society, on which occasion I also gave a demonstration dealing with this subject. Three sets of observers had therefore been working independently on it.

As said above, the process was first observed during the measurement of the total oxygen capacity of anæmic rabbits' blood.

It could clearly and easily be shown that the process of oxygen consumption took place in the plasma (or serum) because it was still evident in serum from which the corpuscles had been separated by centrifugalisation, the blood having been defibrinated by whipping.

A word here about a line of investigation which led only to negative results. It was possible that in anæmia glutathione could appear in plasma as an autoxidisable substance. It is well known that this substance does not occur in normal plasma, but we thought it might occur under abnormal circumstances such as anæmia. Our reason was that anæmia, induced by hæmorrhage, is accompanied by the inflow of tissue fluid into the blood. We therefore applied the nitroprusside test to whole serum as well as to serum filtrates, after having removed the proteins with trichloroacetic acid. We were never able to find more than the slightest traces due certainly only to the small amounts of glutathione set free through the hæmolysis of red cells. The nitroprusside test remained negative even in the most severe degrees of anæmia we obtained, with hæmoglobin values under 20 p.c. and a high degree of lipæmia.

We turn now to the investigation of the process mentioned above with the differential manometer.

*Method.* Two c.c. of ammonia solution (Barcroft(10)) were put in each bottle, and to compensate the volume of serum used (mostly 1 c.c.) that amount of water was put in the second bottle of the manometer. After the potassium ferricyanide (four drops of a saturated solution) had been poured over the alkaline serum, the apparatus was shaken by a mechanical device for several hours in a water bath. At constant time intervals readings were taken.

*Results.* Our first task was to make quantitative observations on the rate of oxidation during progressing anæmia. A rabbit was bled daily, its blood defibrinated by whipping and centrifuged and 1 c.c. serum used for the determination of the  $O_2$  consumption rate. Fig. 1 and Table I give the results of this experiment and show the rising of the oxygen consumption curve from that of the normal animal, to that of the anæmic one. The large oxygen consumption during anæmia is therefore merely an exaggeration of something normally taking place in the rabbit. The

TABLE I.

c.c. O<sub>2</sub> absorbed per 100 c.c. of whole serum or extracted serum respectively.

Date	Feb.	Feb.	Feb.	Feb.		Feb.		Feb.	
1927	5th	7th	8th	9th		10th		11th	
Time	Whole serum	Whole serum	Whole serum	Whole serum	Ex-tracted serum	Whole serum	Ex-tracted serum	Whole serum	Ex-tracted serum
15	0.13	—	—	—	—	—	—	—	—
30	0.54	1.02	1.36	1.08	—	7.40	0.86	—	—
45	—	—	—	—	—	—	—	—	—
1 hr. 60	—	1.78	2.95	4.42	—	—	—	—	—
15	—	2.06	—	—	—	—	—	—	—
30	—	—	4.04	—	—	—	—	—	—
45	3.67	—	—	—	—	—	—	—	—
2 hr. 60	—	—	—	—	—	—	—	33.5	4.87
15	—	—	—	—	—	—	—	—	—
30	—	—	—	—	1.2	—	—	38.7	5.75
45	—	—	—	—	—	—	—	—	—
3 hr. 60	—	—	—	—	—	33.80	5.86	—	6.52
15	7.09	3.23	—	—	—	—	—	—	—
30	—	—	6.69	12.44	—	36.07	6.69	—	—
45	7.69	4.11	—	—	—	—	—	—	—
4 hr. 60	7.97	—	7.01	—	—	38.03	7.48	—	—
15	—	4.81	—	—	—	—	—	—	—
30	—	—	7.27	—	—	39.65	8.01	—	—
45	—	—	—	—	1.87	40.40	8.41	—	—
5 hr. 60	8.95	—	7.80	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—
30	—	5.09	7.82	—	—	—	—	—	—
45	—	—	—	16.76	—	—	—	—	—
6 hr. 60	—	—	—	—	—	—	—	—	—

oxygen consumption, after six days of bleeding, reached a value of about 40 c.c. of O<sub>2</sub> per 100 c.c. of serum in 2.45 hr.

Although no quantitative estimate of the lipoids in the serum was made, a lipæmia was observed, progressing with the anæmia; for the serum, at first quite clear and transparent in the normal animal, became opalescent and finally milky in the anæmic. This visible lipæmia increased with the increase of the O<sub>2</sub> consumption. The same results were secured with oxalated as well as with fluoride plasma; oxalate and fluoride therefore do not interfere with the process of autoxidation.

Nevertheless, however severe the anæmia, or however manifest the lipæmia, no oxygen consumption could be observed as long as the alkaline solution of serum alone was shaken without adding the potassium ferri-cyanide. This substance seems to play the rôle of a catalyst. To test the possibility of a catalysis of the autoxidation of unsaturated fatty acids in the lipæmic serum by a metallic ion, analogous to the oxidation of lecithin in the presence of iron (Thunberg(11)), or of some other substances

importance of the proteins. Two communications by Parsons(8), (9), were made on May 14th to the Physiological Society, on which occasion I also gave a demonstration dealing with this subject. Three sets of observers had therefore been working independently on it.

As said above, the process was first observed during the measurement of the total oxygen capacity of anæmic rabbits' blood.

It could clearly and easily be shown that the process of oxygen consumption took place in the plasma (or serum) because it was still evident in serum from which the corpuscles had been separated by centrifugalisation, the blood having been defibrinated by whipping.

A word here about a line of investigation which led only to negative results. It was possible that in anæmia glutathione could appear in plasma as an autoxidisable substance. It is well known that this substance does not occur in normal plasma, but we thought it might occur under abnormal circumstances such as anæmia. Our reason was that anæmia, induced by hæmorrhage, is accompanied by the inflow of tissue fluid into the blood. We therefore applied the nitroprusside test to whole serum as well as to serum filtrates, after having removed the proteins with trichloroacetic acid. We were never able to find more than the slightest traces due certainly only to the small amounts of glutathione set free through the hæmolysis of red cells. The nitroprusside test remained negative even in the most severe degrees of anæmia we obtained, with hæmoglobin values under 20 p.c. and a high degree of lipæmia.

We turn now to the investigation of the process mentioned above with the differential manometer.

*Method.* Two c.c. of ammonia solution (Barcroft(10)) were put in each bottle, and to compensate the volume of serum used (mostly 1 c.c.) that amount of water was put in the second bottle of the manometer. After the potassium ferricyanide (four drops of a saturated solution) had been poured over the alkaline serum, the apparatus was shaken by a mechanical device for several hours in a water bath. At constant time intervals readings were taken.

*Results.* Our first task was to make quantitative observations on the rate of oxidation during progressing anæmia. A rabbit was bled daily, its blood defibrinated by whipping and centrifuged and 1 c.c. serum used for the determination of the  $O_2$  consumption rate. Fig. 1 and Table I give the results of this experiment and show the rising of the oxygen consumption curve from that of the normal animal, to that of the anæmic one. The large oxygen consumption during anæmia is therefore merely an exaggeration of something normally taking place in the rabbit. The

TABLE I.

c.c. O<sub>2</sub> absorbed per 100 c.c. of whole serum or extracted serum respectively.

Date	Feb.	Feb.	Feb.	Feb.	Feb.	Feb.	Feb.	Feb.	Feb.
1927	5th	7th	8th	9th	10th	10th	11th	11th	11th
Time	Whole serum	Whole serum	Whole serum	Whole serum	Ex-tracted serum	Whole serum	Ex-tracted serum	Whole serum	Ex-tracted serum
15	0.13	—	—	—	—	—	—	—	—
30	0.54	1.02	1.36	1.08	—	7.40	0.86	—	—
45	—	—	—	—	—	—	—	—	—
1 hr. 60	—	1.78	2.95	4.42	—	—	—	—	—
15	—	2.06	—	—	—	—	—	—	—
30	—	—	4.04	—	—	—	—	—	—
45	3.67	—	—	—	—	—	—	—	—
2 hr. 60	—	—	—	—	—	—	—	33.5	4.87
15	—	—	—	—	—	—	—	—	—
30	—	—	—	—	1.2	—	—	38.7	5.75
45	—	—	—	—	—	—	—	—	—
3 hr. 60	—	—	—	—	—	33.80	5.86	—	6.52
15	7.09	3.23	—	—	—	—	—	—	—
30	—	—	6.69	12.44	—	36.07	6.69	—	—
45	7.69	4.11	—	—	—	—	—	—	—
4 hr. 60	7.97	—	7.01	—	—	35.03	7.48	—	—
15	—	4.81	—	—	—	—	—	—	—
30	—	—	7.27	—	—	39.65	8.01	—	—
45	—	—	—	—	1.87	40.40	8.41	—	—
5 hr. 60	8.95	—	7.80	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—
30	—	5.09	7.82	—	—	—	—	—	—
45	—	—	—	10.76	—	—	—	—	—
6 hr. 60	—	—	—	—	—	—	—	—	—

oxygen consumption, after six days of bleeding, reached a value of about 40 c.c. of O<sub>2</sub> per 100 c.c. of serum in 2.45 hr.

Although no quantitative estimate of the lipoids in the serum was made, a lipæmia was observed, progressing with the anæmia; for the serum, at first quite clear and transparent in the normal animal, became opalescent and finally milky in the anæmic. This visible lipæmia increased with the increase of the O<sub>2</sub> consumption. The same results were secured with oxalated as well as with fluoride plasma; oxalate and fluoride therefore do not interfere with the process of autoxidation.

Nevertheless, however severe the anæmia, or however manifest the lipæmia, no oxygen consumption could be observed as long as the alkaline solution of serum alone was shaken without adding the potassium ferri-cyanide. This substance seems to play the rôle of a catalyst. To test the possibility of a catalysis of the autoxidation of unsaturated fatty acids in the lipæmic serum by a metallic ion, analogous to the oxidation of lecithin in the presence of iron (Thunberg(11)), or of some other substances

of the type of linoleic acid by Mn or Co, we tried the influence of  $\text{FeCl}_3$  in two concentrations, namely four drops of a 10 p.c. and the same amount of a 0.01 p.c. solution. The ferric ion had no influence, and no oxygen consumption could be observed during five hours of shaking. Next a complex ion containing iron in a masked form, namely potassium ferrocyanide (four drops of a sat. sol.), was used with the same negative effect. It appeared therefore that in the absence of ferricyanide no oxidation takes place by itself, and that this ion is intimately related to the  $\text{O}_2$  consumption being probably more than a mere catalyst. The influence of hæmoglobin (see Robinson<sup>(12)</sup>) as a catalyst of this process will be referred to at the end of this paper.

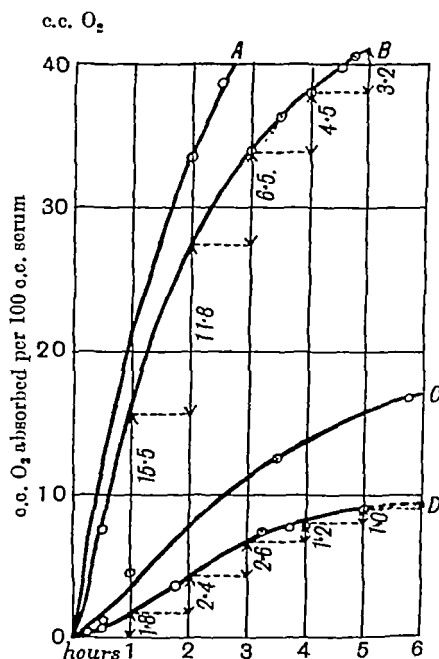


Fig. 1. Absorption of oxygen in c.c. A, B, C and D curves respectively determined on 5th (normal), 9th, 10th, 11th February, 1927.

From the analysis of the curves of Fig. 1 a brief attempt will be made to study the speed of the reaction. If one plots, instead of the total oxygen consumed after an interval of time, the oxygen consumed per unit of time (per hour), one obtains the curves in Fig. 2 derived from those of Fig. 1. Fig. 2 shows the rate of oxidation per hour. The maximum oxygen consumption occurs during the first few hours, falling

afterwards, the maximum being reached much earlier with increased lipæmia. With a very lipæmic serum it is attained in less than an hour,

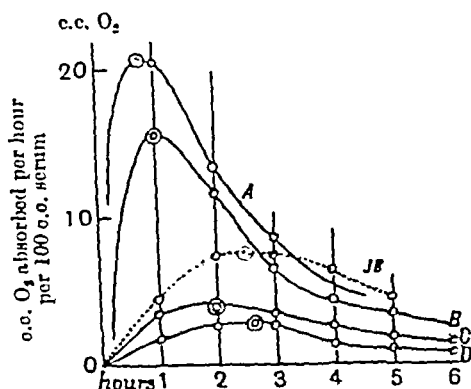


Fig. 2. Rate of absorption in c.c. O<sub>2</sub> per hour calculated from 15 min. determinations. A, B, C and D as above.

on the other hand much later (2-3 hr.) in sera poor in lipoids. The only figure which can be given as an index of this degree of oxidation in sera of different lipid concentrations is the total quantity of oxygen consumed when the reaction is complete. But the end point of the O<sub>2</sub> consumption is only reached after a long time, and for the lecithin + FeCl<sub>3</sub> system Thunberg(11) is doubtful as to whether an equilibrium is ever reached at room temperature. In our case nine days were not sufficient to reach this end point at room temperature (without shaking). Considerable difficulties therefore are connected with the quantitative analysis of this phenomenon, more so as dilution of sera alters the rate of O<sub>2</sub> absorption as also does a change of temperature.

Fig. 3 shows the oxygen consumption curves of whole serum and of the same serum deprived as far as possible of its lipoids by ether extraction. The dotted lines represent the oxygen consumption curves of sera thus treated. One can see that the O<sub>2</sub> uptake is lowered by the removal of the greater part of the lipoids to a value varying between  $\frac{1}{6}$  and  $\frac{1}{8}$  of the original amount, this lowering being dependent on the thoroughness of the extraction. Even after three prolonged ether extractions the serum does not entirely lose its power of auto-oxidation, owing either to the impossibility of extracting all the lipoids with ether alone, or to the presence of some other components still in the serum, proteins for instance, as already pointed out by Douglas(7) and Parsons(8), (9).

The ether extract itself (containing the extracted fats) showed a very low consumption of O<sub>2</sub>, and even if the ether was removed by a vacuum



pump at low temperature to prevent as far as possible oxidation of the unsaturated unstable fatty acids, auto-oxidation of the extracted lipoids

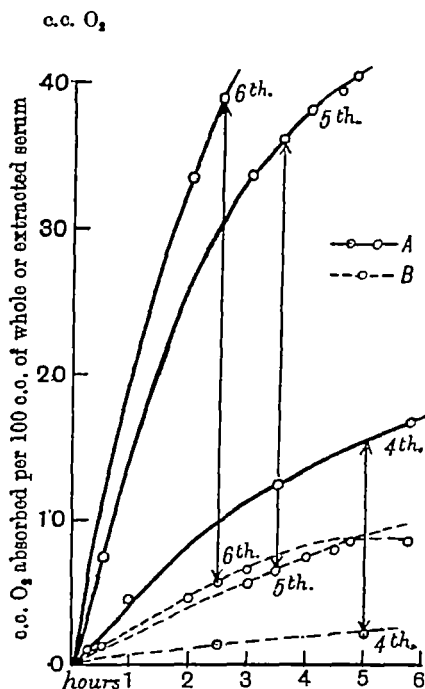


Fig. 3.

Fig. 3. Oxygen absorption in c.c. on 4th, 5th and 6th day of anemia in A by normal serum (firm line), and B serum from which lipoids have been extracted as far as possible with ether (broken line).

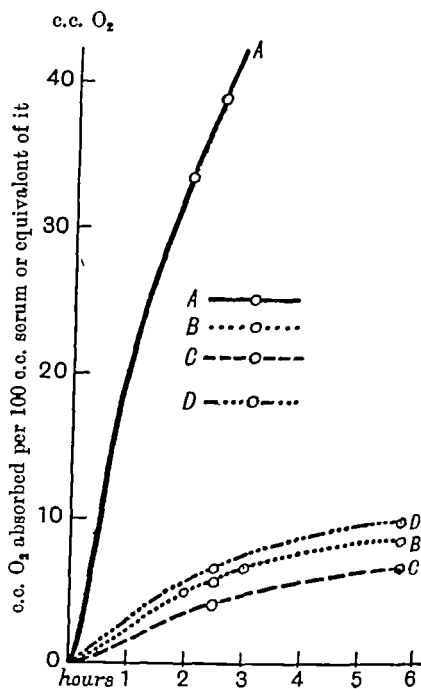


Fig. 4.

Fig. 4. Oxygen absorption. A, whole serum; B, serum treated with ether; C, ethereal extract; D, extracted serum and ethereal extract.

in the presence of ferricyanide was not improved, as shown in Fig. 4, where, besides the curve of the O<sub>2</sub> uptake of the whole serum, that of the extracted serum (freed from lipoids) and that of the ether extract (containing the lipoids) is given. Adding the fats (ether extract) again to the serum freed from lipoids a stronger oxygen consumption is obtained, but far less than before the separation (see Fig. 4).

The fact that the lipid extract shows such a reduced oxygen consumption suggested several possibilities: namely (a) that the fats were oxidised during their separation from the plasma and the evaporation of the ether; (b) that the dispersed phase of our lipid emulsion or suspension was much more coarse than that which pre-existed in the whole serum,

the active surface being decreased; (c) that some other substances contained in the serum are closely connected with the lipoids in the production of this phenomenon.

Here we wish to draw attention to a strange observation, abstaining for the present from any explanation.

If one shakes an active lipæmic serum with ether, and if afterwards, without separating the ether from the serum, one evaporates the latter under low pressure (the ether, containing the lipoids, remaining during the whole evaporation in contact with the extracted serum), the serum thus obtained does not lose appreciably its auto-oxidation power, although the dispersion seems to be quite changed as can be seen by the intense opacity of the serum thus treated. This experiment seems to be in contradiction with the last one mentioned.

During the evaporation ether, from which the peroxidises were removed, was used in order to avoid as much as possible the oxidation of the lipoids during the extraction and especially during the subsequent evaporation. Low pressure and low temperature, as already stated, were also resorted to for the same purpose.

Different devices were tried to increase the active surface of our extracted lipoids such as grinding with charcoal and evaporation on filter paper. The latter only was successful (see Fig. 5), but even here,

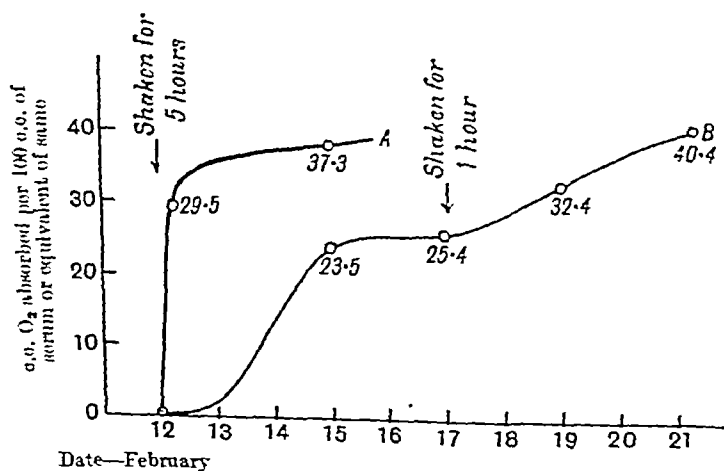


Fig. 5. Oxygen absorption. A, whole serum; B, ethereal extract on blotting paper.

while the whole serum showed after five hours of shaking an absorption of 29.5 c.c. O<sub>2</sub> p.c., the extracted lipoids showed after this time only traces

of  $O_2$  absorption, which increased however the next and following days to degrees we never observed before. An induction period was here manifest, but the long time during which the experiment had run (nine days) makes it open to criticism as to bacterial growths.

Further, evidence was obtained that light was not necessary as it is known to be in the oxidation of unsaturated fatty acids.

A word should also be said about other serum constituents which, as already mentioned, might play an important part, namely the proteins and the soluble organic compounds such as glucose, amino-acid, uric acid, urea, etc. We found it rather difficult to precipitate the proteins alone without removing at the same time the amphoteric and colloidal phosphatides which, as shown, play such an important part (see Parsons (8), (9)), secondly, serum filtrates obtained after the precipitation of the proteins and lipoids with trichloroacetic acid are quite inactive with regard to oxygen consumption in the presence of potassium ferricyanide in an acid medium. It follows therefore that the substances contained in the filtrate, like amino-acids, creatine, uric acid, urea, glucose, etc. are, if deprived of the colloidal system lipoids-proteins, inactive by themselves; but on the other hand the possible influence of proteins beside the lipoids cannot be excluded because they were precipitated simultaneously with the lipoids.

We turn once more to the system lipoids-ferricyanide. From the present series of experiments it was difficult to decide the part played by each of these two components in the  $O_2$  consumption. As traces of ferricyanide did not catalyse the process and as apparently no other common catalyst could be found, it is possible that ferricyanide might be changed as well as the lipoids.

An attempt was also made to oxidise the unsaturated fatty acids of the lipoids by shaking the serum with hydrogen peroxide or with iodine solution before the addition of ferricyanide, but these did not influence the later  $O_2$  uptake in the presence of ferricyanide.

Finally an attempt to measure quantitatively the degree to which dilution of lipæmic serum or of ferricyanide influenced the oxygen uptake was made. Fig. 6, derived from Table II, shows such oxygen consumption curves:

- (1) Using 1 c.c. of a very active lipæmic serum to which four drops of ferricyanide solutions of different molar concentration was added;
- (2) Using 1 c.c. of sera of different dilutions to which four drops of a nearly one molar ferricyanide solution was added.

The curves obtained are far from being strictly quantitative, firstly

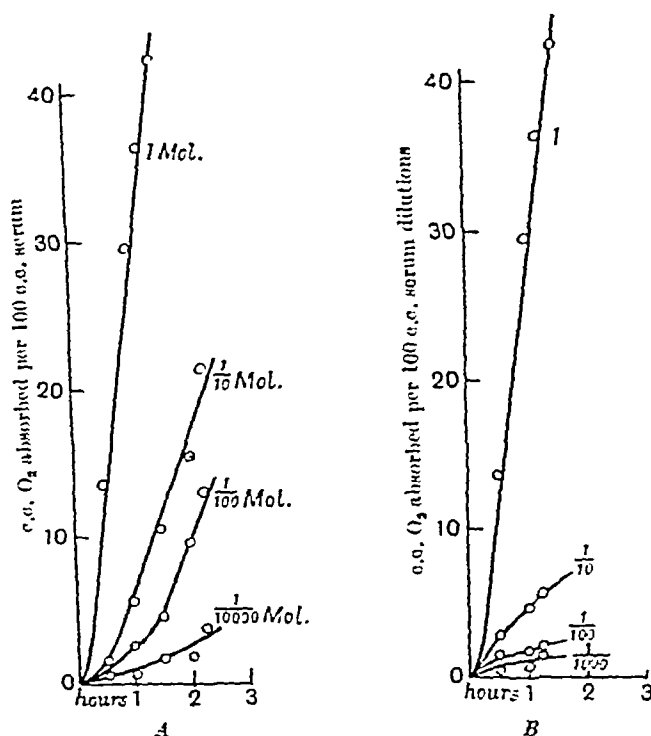


Fig. 6. Oxygen absorption per 100 c.c. A, with various dilutions of ferricyanide; B, with various dilutions of serum.

TABLE II.

Date, 1927 June 29th and May 2nd	c.c. O <sub>2</sub> absorbed per 100 c.c. serum							
	Different potassium ferricyanide dilutions (used 4 drops)				Different serum dilutions (used 1 c.c.)			
	1 mol.	1/10 mol.	1/100 mol.	1/10000 mol.	un-diluted	1/10	1/100	1/1000
	Time							
1 hr.	15	—	—	—	13.8	2.85	1.05	0.15
	30	13.8	1.36	0.31	0.43	—	—	—
	45	—	—	—	—	—	—	—
	60	29.8	5.76	2.31	0.53	29.8	4.64	1.45
2 hr.	15	36.3	—	—	36.3	5.44	1.46	1.10
	30	42.3	10.36	4.61	1.79	42.3	—	—
	45	—	—	—	—	—	—	—
	60	—	15.36	9.61	1.80	—	—	—
25 hr.	15	—	21.36	12.91	3.80	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
Experiment continued without shaking.								
25 hr.	—	—	—	—	59.7	12.5	2.9	2.07
26 hr.	59.8	31.9	18.48	9.6	—	—	—	—

on account of the change of the oxidation rate which, as already stated, changes with the lipid concentration, and secondly, because with the apparatus used it was impossible to pour quantitatively the four drops of ferricyanide into the serum. In spite of this error the curves show that the lipoids play quantitatively a much more important part in the oxidation process, which is more affected by a dilution of the serum (consequently of the lipoids) than by the same dilution of the ferricyanide; in any case the lowering of the oxygen uptake bears no direct relation to the dilution of serum or ferricyanide. From Table II it can be seen that after 26 hours the dilution of the ferricyanide from molar to  $\frac{1}{10}$  molar is accompanied by a lowering of the oxygen uptake to the half, namely from 59.8 p.c. to 31.9 p.c., whereas the same dilution of the serum to  $\frac{1}{10}$  is accompanied by a fivefold lowering, namely from 59.8 p.c. to 12.5 p.c. The intimate relation of both substances has therefore once more to be admitted.

The fact mentioned above, and especially that communicated by Parsons that permanganate acts in the same way as ferricyanide in the process of oxygen absorption, argues in favour of the following explanation, namely that ferricyanide plays the rôle of an oxidative agent transferring foreign oxygen to the lipoids whilst being itself probably reduced.

It was stated elsewhere that oxygen consumption occurs even in serum of normal rabbits, and is thus normal and physiological. It could be shown further that the normal circulating lipoids of the serum were the cause of this process which was very much diminished after ether extraction of the serum.

We repeated our experiments on human blood. It showed quite the same properties as rabbits' blood. This phenomenon seems to be quite general as far as the blood of two species can be relied upon, being only exaggerated in pathological conditions accompanied by lipæmia.

The dependence of this reaction upon a state of lipæmia could further be observed in post-absorptive lipæmia. The  $O_2$  consumption of normal human serum in presence of ferricyanide is much more marked after fat absorption from the intestine which follows a meal, as can be seen from Table III and Fig. 7. The  $O_2$  consumption curve of G.L. is given before and after a breakfast containing fat, the latter being much higher than the former. The serum after breakfast was opalescent: that of J.B. in the same figure was slightly milky after breakfast.

As a practical consequence it must be stated (1) that no accurate results can be secured in states of lipæmia with the ferricyanide method, (2) that the more rapid the analysis the more accurate the results obtained.

TABLE III.

		c.c. O <sub>2</sub> absorbed per 100 c.c. serum			
Date, 1927	Time	Feb. 8th After breakfast J.B.	Feb. 7th After breakfast G.L.	March 5th	
				Before breakfast G.L.	After breakfast G.L.
	15	—	—	—	—
	30	1.75	0.17	0.02	1.45
	45	—	—	—	—
1 hr.	60	4.36	1.76	—	—
	15	—	2.69	—	—
	30	7.32	—	—	—
	45	—	—	—	—
2 hr.	60	—	—	—	—
	15	—	—	—	—
	30	—	—	3.95	9.68
	45	—	—	—	—
3 hr.	60	—	—	—	—
	15	—	10.13	—	—
	30	22.32	—	—	—
	45	—	11.03	—	—
4 hr.	60	25.02	—	—	—
	15	—	12.99	—	—
	30	27.50	—	—	—
	45	—	14.32	—	—
5 hr.	60	29.57	—	—	—
	15	—	15.65	—	—
	30	31.35	16.27	—	—
	45	—	—	—	—
6 hr.	60	—	—	—	—

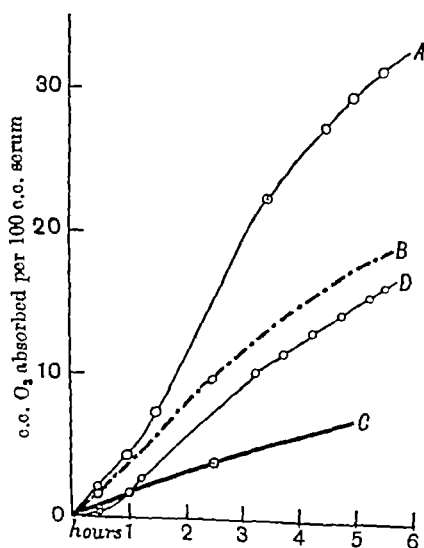


Fig. 7. Oxygen absorption of serum from A, J.B. after breakfast, February 8th; B, G.L. after breakfast, March 5th; C, G.L. before breakfast, March 5th; D, G.L. after breakfast, February 7th.

Having shown the part that the lipoids play in this oxidation process, we turn back to a substance already mentioned as a possible catalyst, namely to hæmoglobin, which, like ferricyanide, contains its iron in a masked form. Miss Robinson<sup>(12)</sup> showed its catalytic action upon unsaturated fatty acids and we applied it therefore in our experiments instead of ferricyanide. Time did not allow us to do more than a few experiments, nor to analyse the problem thoroughly, but we think nevertheless the experiments are of some interest.

The first experiment was made on June 4th, 1927, using in one of the bottles of the differential manometer 1 c.c. of anæmic and lipæmic whole blood hæmolysed by adding 2 c.c. of ammonia, in the second bottle water was put to compensate for the volume of blood. After one hour's shaking, during which the  $O_2$  absorption was 1.79 c.c. per 100 c.c. of blood, the oxygen absorption rose during the following 18 hr. without any shaking to 19 c.c. per 100 c.c. of blood. Because this experiment was open to criticism a second experiment was made in the following way using instead of ferricyanide a concentrated hæmoglobin solution. In one of the bottles was 1 c.c. of lipæmic serum, to which 1 c.c. of a 50 p.c. hæmoglobin solution (Haldane hæmoglobinometer) and 1 c.c. of ammonia solution were added, in the second bottle containing the same amount of hæmoglobin solution the serum volume was compensated with water.

After 15 min. of shaking during which only traces of oxygen were absorbed an  $O_2$  consumption of 12 c.c. per 100 c.c. of serum followed in the next 18 hr. and 26 c.c. after 42 hr. without any shaking. No ferricyanide was added in either of these two experiments. It should be noted that the hæmoglobin solution was bright red in the control bottle, without serum, whereas in that containing serum its colour was dark red.

Finally an experiment showed that Hb activates the nearly inactive lipoids contained in an ether extract.

We may therefore conclude there is an analogy between potassium ferricyanide and hæmoglobin in respect of their action as oxidative agents and the probable rôle of their masked iron.

From our experiments it is therefore obvious that this phenomenon must occur in all cases of lipæmia, physiological as well as pathological, through the interaction of hæmoglobin and lipoids, and must be directly connected with the lipoids, that is the phosphatides which are known to be increased in alimentary lipæmia.

The oxygen uptake may be due partly to the oxidation of the unsaturated fatty acids, free or contained in the phosphatides, the de-

saturation of the saturated stored fatty acids being the first stage of fat catabolism and preceding their further oxidation (Pryde<sup>(13)</sup>).

With the possibility that hæmoglobin acts in the same way as ferricyanide the importance of this reaction increases. hæmoglobin being a physiological substance occurring in blood as well as in the tissues and liver, where an important part of fat catabolism takes place normally and under pathological conditions.

From Miss Robinson's<sup>(12)</sup> results, A. V. Hill<sup>(14)</sup> makes some interesting remarks as to a third hæmoglobin function, namely as an oxidative catalyst in the tissues, which is of another type from its well-known reversible combination with  $O_2$ . It seems therefore that in lipæmia this special third function of hæmoglobin may be increased in the blood.

Of further interest are the changes which the hæmoglobin molecule might itself suffer during this oxidation process which occurs in the presence of active lipoids in the tissues. It is therefore possible that the lipoids play an important rôle in the catabolism of hæmoglobin.

Further investigation, besides clearing up the puzzle as to the part which the lipid-protein complex on the one hand and the hæmoglobin or ferricyanide on the other play in this process, may enlarge our knowledge of lipæmia and also of the biological significance of the oxidisable substances present in the blood in this condition and in varying amounts in others, normal and pathological, such as arteriosclerosis, lues, nephrosis, diabetes, etc.

Finally new light may be thrown upon the catabolism of the hæmoglobin molecule itself and the influence which the lipoids have on this catabolism in normal life, and also in such diseases as pernicious anæmia and hæmolytic jaundice.

#### SUMMARY.

- (1) The system consisting of a mixture of plasma (or serum) and potassium ferricyanide absorbs oxygen when exposed to air.
- (2) In this reaction the ferricyanide cannot be replaced by ferric chloride or by ferrocyanide; plasma in the presence of these substances does not absorb oxygen from the air. Hæmoglobin however has an action similar to ferricyanide.
- (3) The facts stated above introduce an error into the estimation of oxygen in blood with potassium ferricyanide. This error is a function of
  - (a) The time taken for the analysis;
  - (b) The concentration of lipoids in the plasma;
  - (c) And, to a less extent, the concentration of ferricyanide.



In the Van Slyke method the time taken is so short as to eliminate the error, and this method is probably the only one suitable for the estimation of the oxygen in lipæmic bloods.

Other methods in which ferricyanide is used are accurate in proportion as they can be manipulated rapidly, which probably gives the differential method an advantage over one in which the errors are not balanced.

Also inasmuch as hæmoglobin can act in a similar way to ferricyanide as a catalyst for the oxidation of fats, even this error may be to some extent, though not entirely, balanced in the differential apparatus.

Because methods involving small quantities of blood are more rapid than those involving large quantities, ferricyanide appears at its best in micrometric apparatus.

(4) The removal of ether soluble lipoids from plasma greatly reduces its power of absorbing oxygen when treated with ferricyanide.

(5) The extracted lipoids when redissolved have some power of absorbing oxygen in contact with ferricyanide, though they have not so great a power as the original plasma from which they were extracted.

(6) Serum filtrates obtained after precipitation of the colloidal complex lipoids and proteins are quite inactive.

(7) The phenomenon which forms the subject of this paper is probably of wide importance and occurs irrespective of whether the lipæmia is caused by digestion of fats or anæmia.

(8) The process must occur in the body wherever hæmoglobin really comes into molecular contact with lipoids.

I am indebted to Professor Barcroft for the interest shown throughout the research as well as to Mr Tunnicliffe for valuable advice.

Part of the expense of the above research (apparatus) was borne by the Medical Research Council and part (chiefly animals) by the Rockefeller Foundation.

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## FURTHER OBSERVATIONS ON PHOSPHAGEN.

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THE collection of experimental results presented in this paper is too inconsecutive to be used as the basis of any theoretical discussion of phosphagen, but the results are published in the hope that they will be of practical use to other workers in the field of muscle chemistry.

Phosphagen is the name given to a substance which is present in considerable quantities in resting voluntary muscle, and is of the nature of an ester of phosphoric acid, remarkable for the ease with which the phosphoric acid is hydrolysed off in dilute acid solution. From the skeletal muscles of rabbits we have isolated a material which shows this typical lability and which is a compound of creatine<sup>1</sup> and phosphoric acid(1). But it is not yet certain whether this substance is identical with, or is a breakdown product of, phosphagen.

The extreme lability of phosphagen in acid solution makes it imperative that its extraction by the following method from the muscle should be completed in the minimum of time. To precipitate proteins in the least bulky and most easily filtrable form, 4 p.c.  $\text{CCl}_3\text{COOH}$  has been found best (40 c.c. per grm. of muscle), filtration being carried out within 12 minutes of the grinding of the muscle. It is not at present possible to discover by direct estimation whether the phosphagen extraction is complete in this time: presumably it is, since the sum of the phosphagen and inorganic phosphate extracted is not increased by longer extraction (up to 24 hours). Neither is there any appreciable increase in the amounts extracted of creatine, lactic acid, or acid-soluble organic phosphorus (Table I).

Even with an extraction as short as 10 minutes there nevertheless occurs some breakdown of the extracted phosphagen in the acid medium. If phosphagen be dissolved in 4 p.c.  $\text{CCl}_3\text{COOH}$  at a dilution comparable to a muscle extract, at room temperature half of it is hydrolysed in

<sup>1</sup> Confirming Fiske and Subbarow (11).

TABLE I.

Time of extraction in minutes ...	10	60	180	1180
Phosphagen P and Inorganic P ...	89	88	87	88
Organic P (other than phosphagen)	69	67	68	72
Lactic acid ... ..	139	133	136	139
Total creatine ... ..	500	490	510	510

Five grm. of muscle were ground up in 200 c.c. of 4 p.c.  $\text{CCl}_3\text{COOH}$  and divided into four portions which were filtered at the stated times. Organic P estimated in duplicate, and lactic acid in triplicate. Results are expressed in mg. per 100 grm. of muscle.

200 minutes. In 10 minutes about 5 p.c. is hydrolysed. It is therefore necessary to apply a correction for this loss, and to correct the value for inorganic phosphate in the opposite sense.

As to the technique of estimating both phosphagen and inorganic phosphate we have found Briggs' method, suitably modified, most useful. The details of the technique we have published previously (2).

With regard to the plotting of the "colour ratios" it is necessary to take readings only in three groups at, say, 3, 6, and 8 minutes from the beginning of the reduction, and a reading at 50 minutes. The reciprocals of the readings lie in a straight line for the first 8 minutes of the reduction, and extrapolation back to zero time is therefore simple and accurate. We have been able to test this method on artificial mixtures of phosphagen and inorganic phosphate, and have obtained perfect results in all cases save where the proportion of phosphagen to phosphate is very low. Thus the method would tend to make a badly fatigued muscle appear even more fatigued (Table II).

TABLE II.

Experiment	...	I	II	III	IV	V
Phosphagen P	{Theoretical	16	32	49	65	81
	{Found	12	31	49	66	81
Inorganic P	{Theoretical	71	53	36	18	0
	{Found	76	55	35	17	0
Total	{Theoretical	87	85	85	83	81
	{Found	88	86	84	83	81

Results expressed in mg. P per 5 litres of solution (actually 5 c.c. were used for each analysis).

It may be mentioned that Meyerhof has elaborated an alternative technique in which the inorganic phosphate is precipitated by an alkaline magnesia mixture and estimated separately. The result subtracted from the total as estimated in the ordinary Briggs' method gives the phosphagen content of the muscle.

*The estimation of free creatine.* In 1911 a method was published by Walpole (3) for the estimation of creatine (as distinct from creatinine) in urine. The method is based on the fact that creatine condenses with

diacetyl in alkaline solution to give a pink coloured product. Several other substances containing the guanidine group<sup>(1)</sup>, including arginine, display the same property. We have found that the creatine combined in phosphagen is not estimated by this method, and have made use of this fact in studying the fate of the phosphagen creatine of the muscle when the phosphagen has been caused to disappear. In its present form the method does not give accurate quantitative results, and the experiments cited in Table III must be taken only as a very rough indication.

TABLE III. Free creatine in frog's gastrocnemius

Experiment	...	...	I	II	III	IV	V
Resting ...	..	...	100	170	210	160	200
Fatigued ...	...	...	430	—	—	370	—
In rigor ...	..	...	—	350	—	—	400
Incubated in $\text{NaHCO}_3$ ..	..	...	—	—	415	—	—
Ditto in presence of NaF	..	...	—	—	400	—	—

The results are expressed as mg. of creatine per 100 gm. of muscle. Creatine estimated by the method of Walpole. (In Exp I the phosphagen P fell from 47 to 8, and in Exp. II from 50 to 0.)

*Comparative study.* In an earlier publication<sup>(2)</sup> we referred to the fact that plain muscle appeared to contain no phosphagen. We have followed up this clue<sup>1</sup> by studying the muscles of a number of animals, with some interesting results. Whilst the voluntary muscles of the vertebrates studied, even as low in the scale as *amphioxus*, all contained phosphagen, we were unable to demonstrate its presence in the muscle of any invertebrate examined. Parallel estimations of the acid-soluble creatine brought out clearly its physiological relationship. Muscles containing no phosphagen contained no acid-soluble creatine (Table IV).

Meyerhof has confirmed this discovery as regards the crab, and has further stated<sup>(5)</sup> that although phosphagen is absent, there is present a similar substance having rather less lability in acid, and (in a recent private communication) probably containing arginine in the place of creatine. In view of the work of Kutscher<sup>(6)</sup>, who found that the muscles of the crayfish were rich in arginine but contained no creatine, it seems possible that this substance isolated by Meyerhof from the crab muscle may be present in the muscles of other invertebrates. It is a curious fact that the colour reaction given by free creatine with diacetyl is given also by arginine. It is possible that the guanidine residue common to these two compounds (and responsible for this colour reaction) may be the reason for their relationship in muscle physiology. In a more complete comparative study of muscles one

<sup>1</sup> Acting at the suggestion of Sir Walter Fletcher, whose helpful interest in this work we gratefully acknowledge.

must be prepared to meet (as Meyerhof has suggested) a series of "phosphagens" serving similar functions in different types of contractile tissue.

TABLE IV. A comparison of different muscles with respect to their content of phosphagen.

	Inorganic P	Phosphagen P	Creatine
VERTEBRATES:			
Snake (dorsal)	65	40	—
Guinea-pig (gastrocnemius)	58	22	—
Dog fish (coraco-mandibular)	51	18	460
Plaice (dorsal)	91	37	—
Cottus (dorsal)	130	13	410
Frog	{ (heart)	20	5
	{ (gastrocnemius)	30	50
	{ (stomach)	20	0
Ray (coraco-mandibular)	50	40	440
Tortoise (hind limb)	64	15	—
Rabbit	{ (gastrocnemius)	26	62
	{ (soleus)	47	32
Amphioxus (whole body)	67	33	420
INVERTEBRATES:			
Lobster (tail)	74	0	0
Crab (claw)	48	0	—
Aplysia (foot)	2	0	—
Pecten (adductor)	114	0	0
Holothuria (longitudinal band)	12	0	—
Mytilus (adductor)	50	0	0
Aurelia (contractile tissue at circumference)	0.7	0	—
Earthworm (whole body)	Trace	0	—

The results are expressed in mg. of P (or of creatine) per 100 gm. of muscle.

*Anaerobiosis.* The coraco-mandibular muscle of the ray (*R. clavata*) is peculiarly suited to certain types of muscle studies, and we take this opportunity of drawing attention to its advantages. It is a flat, straight-fibred muscle which can be dissected from the fish without any injury. In the adult the muscle weighs up to 20 gm., and is 8–10 cm. long. When cut up into several samples the distribution of phosphate, lactate, etc. is found to be very constant. We have used this muscle to study the effect of resting anaerobiosis on the phosphagen. Cut up into a dozen pieces and kept in an atmosphere of hydrogen the muscle lost most of its phosphagen during the first hour or two, the curve of breakdown resembling a negative exponential. During the first 6 hours the lactic acid production was practically linear, at the rate of about 70 mg. p.c. per hour. But the fact which makes these experiments of special significance is that until 80–90 p.c. of the phosphagen has disappeared the inorganic phosphate production can be ascribed entirely to phosphagen breakdown. Expressed in another way the sum of inorganic phosphate and phosphagen phosphate is constant until the muscle has passed beyond

the physiological range of anaerobiosis. Fig. 1 gives the results of one of five entirely concordant experiments of this nature. A difference is here

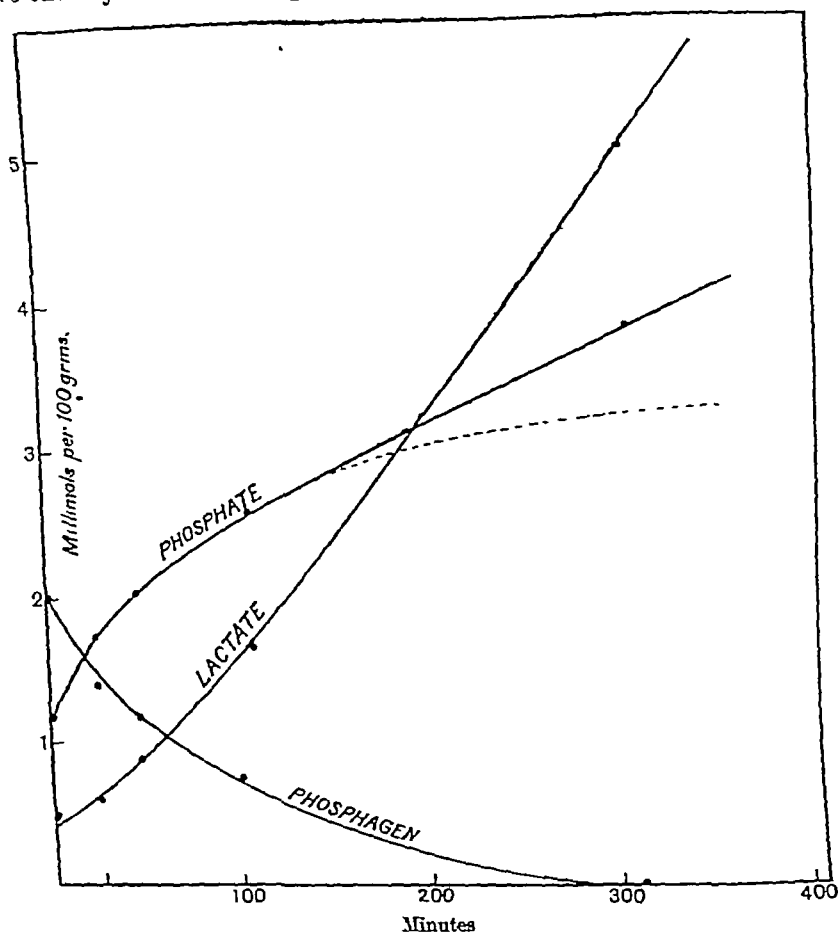


Fig. 1. The coraco-mandibular muscle of a ray (*R. clavata*) was cut into six pieces and allowed to remain in hydrogen at 16° C. One piece was taken for analysis at each of the stated times. The dotted line is the curve of phosphate production which would have been given had the phosphate been derived solely from phosphagen. At the end of 24 hours the last sample contained 700 mg. p.c. lactate, and 162 mg. p.c. phosphate (as P).

observed between the breakdown of phosphagen in resting anaerobiosis and its breakdown in muscular fatigue<sup>1</sup>. In the latter case (Table V) the inorganic phosphate produced accounts for only 60–80 p.c. of the

<sup>1</sup> The disappearance of phosphagen in activity is about 100 times as rapid as in resting anaerobiosis.

phosphagen removed, the remainder being "stabilised," and in the subsequent oxidative recovery of the muscle the fraction of the phosphagen which has been "hydrolysed" is rebuilt before the fraction which has been "stabilised." Indeed we have not so far in isolated muscles found any sign of "unstabilisation" of the "stabilised" fraction. It is to be observed also that the production of lactic acid in anaerobiosis is not directly related to the phosphagen breakdown. We have shown that this is true also in muscular activity, and Meyerhof has reached the same conclusion. When one considers also that the resynthesis of phosphagen during oxidative recovery is nearly complete when the lactic acid removal is only just begun, it is clear that one is dealing with a mechanism chemically distinct from, though perhaps physiologically related to, the production of lactic acid.

TABLE V. The fate of phosphagen phosphorus in muscular activity.

	Phos- phagen P		Inor- ganic P		Or- ganic P		Total		Change due to activity			
	R	F	R	F	R	F	R	F	Phos- phagen P	Inor- ganic P	Or- ganic P	Total
A	61	10	32½	74½	80	89	173½	173½	-51	+42	+9	0
B	50	6	36	75	84½	90	170½	171	-44	+39	+5½	+½
C	41½	15½	43½	60½	76½	85	161½	161	-26	+17	+8½	-½
D	55	45	34½	32½	73½	84½	163	162	-10	-2	+11	-1
E	70	41½	23	45	76	81½	169	168	-28½	+22	+5½	-1
F	45	26	37	49	72	82	154	157	-19	+12	+10	+3

These are six out of a large number of experiments in which one gastrocnemius (F) of a frog was caused to contract isometrically before being analysed. The second gastrocnemius was used as the resting control (R). The measurements of lactic acid, tension, and length of muscle have been omitted from the table. The results are expressed in mg. of P per 100 grm. of muscle. In Exps. C, D, and E the muscles were tetanised isometrically, and in the others a series of twitches at one second intervals were given.

*The influence of the fluoride ion.* In a previous communication to this *Journal*(9), we referred to the curious effect of fluoride on the phosphagen in a minced muscle preparation suspended in a bicarbonate buffer. Whether fluoride was present or not, all the phosphagen disappeared, but in the presence of fluoride this disappearance did not result in the liberation of free phosphate. In Table VI we have collected together a number of experiments bearing on this phenomenon.

This behaviour of phosphagen accounts in part for the so-called "synthetic" effect of the fluoride ion discovered by Embden and Lehnartz(7). The "synthesis" observed by these authors was in the main a conversion of phosphagen phosphorus (which they estimated as inorganic phosphorus) into some acid-stable phosphoric ester. When phosphagen is present some true synthesis can occur however, for added inorganic phosphate disappears to a small extent under the action of

TABLE VI. The effect of fluoride on the fate of phosphagen phosphorus in the incubation of chopped muscle in  $\text{NaHCO}_3$  buffer.

		Initial condition of muscle		After incubation in buffer	
		Phosphagen	Inorganic	(a) No NaF Inorganic	(b) In $M/10$ NaF Inorganic
1	Resting	45	28	91	25
2	"	41	42	—	26
3	"	47	33	—	20
4	"	—	—	115	21
5	"	50	40	—	28
6	Fatigued*	—	56	—	20
7	" *	—	53	—	20
8	{ Resting	—	—	—	21
	{ Fatigued*	—	—	—	21

\* Fatigued by 3' isotonic tetanus.

In these experiments frog gastrocnemii were incubated for 3 hours at  $38^\circ$  in 2 p.c.  $\text{NaHCO}_3$  buffer (after which time no phosphagen remains). The figures represent mg. of P per 100 gm. of muscle.

fluoride. Deuticke(s) has shown that muscle in rigor mortis (which incidentally contains no phosphagen) fails to give the "synthetic" effect. The same is true also of vertebrate involuntary muscle (Table VII), although the typical effect of the fluoride in inhibiting glycolysis of the organic esters is observable.

TABLE VII. The effect of fluoride on the inorganic phosphate formation when chopped plain muscle (frog's stomach) is incubated in  $\text{NaHCO}_3$  buffer.

Inorganic phosphate in muscle			
	Initially	After incubation in $\text{NaHCO}_3$	After incubation in $\text{NaHCO}_3$ and $M/9$ NaF
I	15	30	25
II	20	41	26
III	20	45	28

In these experiments as in those of Table VI the chopped muscle was incubated in 2 p.c.  $\text{NaHCO}_3$  solution for 2 hours, but in this case added fluoride has merely inhibited glycolysis.

A further distinction between plain and voluntary muscle is that added inorganic phosphate is not esterified by the former when the chopped muscle is incubated in the presence of fluoride.

It is safe to deduce from the experiments already quoted in this paper that the phosphate radicle of phosphagen is made use of by the muscle (when stimulated to activity, or when incubated in the presence of fluoride) to esterify some organic compound. From the constancy of the "total acid-soluble phosphorus" figures in Tables V and VIII<sup>1</sup> it is clear that the phosphoric ester produced is a member of the "acid-

<sup>1</sup> The results in Table VIII are a confirmation of those of Wechsellaermann (10).



TABLE VIII. The effect on the total acid-soluble phosphorus of incubation of frog's gastrocnemius in bicarbonate buffer with and without fluoride (at 38°).

	Inorg. P and phosph. P	Acid-sol. org. P other than phosph. P	Total acid- sol. P	Change in		
				Inorg. and phosph. P	Org. P	Total acid- sol. P
<i>Without fluoride:</i>						
Unincubated	76	75	151			
Incubated	128	21	149	+52	-54	-2
Unincubated	75	77	152			
Incubated	137	13	150	+62	-64	-2
<i>With fluoride:</i>						
Unincubated	90	70	160			
Incubated	77	79	156	-13	+9	-4
Unincubated	84	70	154			
Incubated	60	94	154	-24	+24	0

Incubation for 18 hours in  $\text{NaHCO}_3$  buffer both with and without fluoride produced large changes in the ratio of inorganic to organic acid-soluble P, but the sum of the two was unaltered. Results expressed in mg. of P per 100 grm. of muscle.

soluble" group, and the question remains whether or not it is of the class designated by Embden as "lactacidogen" (i.e. the ester or esters hydrolysed rapidly by the muscle enzymes when the minced muscle is incubated in a bicarbonate buffer). The experiments cited in Table IX

TABLE IX. The nature of the phosphoric ester formed as a result of activity.

	Inorg. and phosph. P	Acid-sol. org. P other than phosph. P	Total acid- sol. P	Change in		
				Inorg. and phosph. P	Org. P	Total acid- sol. P
<i>I. Killed immediately:</i>						
Resting	88½	66	154½			
Fatigued	80½	75	155	-8	+8½	+½
<i>Incubated:</i>						
Resting	104½	42½	147			
Fatigued	105½	43½	149	+1	+1	+2
<i>II. Killed immediately:</i>						
Resting	85	76	161			
Fatigued	65	93	158	-20	+17	-3
<i>Incubated:</i>						
Resting	116	39½	155½			
Fatigued	115½	39	154½	-½	-½	-1

In Exp. I one gastrocnemius was tetanised for 2 minutes isotonicly under zero load, and the other gastrocnemius was a resting control. In Exp. II the stimulated gastrocnemius was tetanised isometrically for 2 minutes under 20-30 grm. initial tension. Each set of values is the mean of three independent results (i.e. six frogs were used for each experiment). The small apparent changes recorded in total acid-soluble P are due to small errors of manipulation (arising usually in the weighing of the muscles). They stand in marked contrast to the large rises in organic P and the compensating falls in "phosphagen and inorganic P" resulting from rapid fatigue. Results expressed in mg. of P per 100 grm. of muscle.

indicate quite definitely that this is so. Although, as a result of fatigue, the acid-soluble organic phosphorus of the muscles was increased by

about 8 mg. p.c. in one case, and 17 mg. p.c. in the other, the amount of acid-soluble organic phosphorus remaining after incubation was the same for both resting and fatigued muscles. The ester formed at the expense of phosphagen phosphorus in activity is therefore Embden's "lactacidogen."

It is in our opinion regrettable that the name "lactacidogen" has been given to a substance which in our experience is not diminished, but actually increased in amount during the contraction of an excised muscle, in which lactic acid appears; and when we identify as "lactacidogen" the substance which, during activity, is formed from phosphagen phosphorus we do not wish to be understood to express the opinion that this substance is really the source of lactic acid during muscular contraction.

The method of estimating "lactacidogen" adopted by Embden is not invalidated by the existence of phosphagen, which alike before and after incubation he has determined as inorganic phosphate, so that the difference is correct. On the other hand, all his observations on changes of inorganic phosphate during activity or in rigor are completely invalidated by the fact that a considerable fraction (in the resting muscle the major portion) of this "inorganic phosphate" is really present in an unstable organic form. In view of this latter fact it is obvious that a number of his conclusions must be revised.

#### SUMMARY.

1. Muscles which are capable of rapid energy output (*e.g.* gastrocnemii of frog or rabbit) are, in their resting condition, richer in phosphagen than muscles intended for lower rates of energy expenditure.

2. The rate of disappearance of phosphagen in a muscle when resting under anaerobic conditions cannot be correlated directly with the rate of production of lactic acid. The phosphagen has diminished to an inappreciable quantity before the lactic acid production has reached a quarter of the value it finally attains.

3. The breakdown of phosphagen, whether as the result of fatigue, or rigor, or incubation of the minced muscle in bicarbonate buffer with or without the addition of fluoride, always results in the liberation of free creatine in an amount roughly corresponding to the phosphagen which has disappeared.

4. When phosphagen is present in a minced muscle which is being incubated in the presence of fluoride, some conversion of inorganic phosphate into organic can sometimes be observed. But in muscles

which contain no phosphagen we have failed so far to observe any such synthetic process.

5. The resynthesis of phosphagen which occurs when a fatigued muscle is allowed to recover is very rapid if the surrounding atmosphere is rich in oxygen (one atmosphere or more). Under these conditions the restitution of phosphagen is far more rapid than the restitution of glycogen from lactic acid. Some resynthesis has been observed by Meyerhof in anaerobic conditions, but this is easily masked by the breakdown which is always occurring in the absence of oxygen, even in a resting muscle.

6. The phosphagen destroyed by muscle when resting in anaerobic conditions, or when incubated in a bicarbonate buffer, is accounted for completely by the inorganic phosphate produced: phosphagen destroyed as the result of activity appears only in part as inorganic phosphate, the remainder being accounted for exactly by an increase in the amount of acid-soluble organic esters of phosphoric acid; phosphagen destroyed during incubation in a bicarbonate buffer in the presence of fluoride is accounted for entirely by the phosphoric esters formed. In all these cases the total acid-soluble phosphorus remains constant.

7. The phosphoric ester produced in fatigue is identical with Embden's "lactacidogen"—i.e. it is hydrolysed rapidly by the muscle enzymes when the chopped muscle is incubated in bicarbonate buffer.

Our warmest thanks are due to Dr E. J. Allen at the Marine Biological Station, Plymouth, for permitting us to do part of this work at his Laboratory, and to him and to Dr C. F. A. Pantin for their generous co-operation in selecting material for the comparative study.

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# THE INFLUENCE OF ACID BASE EQUILIBRIUM ON THE ACTIVITIES OF BLOOD VESSELS.

By R. J. S. McDOWALL.

*(From the Department of Physiology, King's College,  
University of London.)*

SINCE Gaskell's<sup>(1)</sup> original observation of the dilatation of blood vessels by the action of acid, the opposite effect, a constriction, has been described commonly to result from the action of alkalies, but in certain conditions also from the application of acids.

In some of the experiments which led to these observations (Fleisch<sup>(2)</sup>, Atzler and Lehman<sup>(3)</sup>), those, namely, in which the method consisted in perfusion of the vessels with fluids at different reactions, the importance of maintaining capillary tone was not appreciated and, as Dale and Richards showed, this tone is rapidly lost in such experiments unless appropriate steps are taken to preserve it.

In others (Evans and Underhill<sup>(4)</sup>, McSwiney and Newton<sup>(5)</sup>) the vessels were merely one of different forms of smooth muscular tissue, the behaviour of which was studied by immersion in fluids at different controlled reactions. McSwiney and Newton in this way found that starting with fluid at  $pH$  7.5 the first changes in the alkaline direction caused contraction, the first changes in the opposite direction relaxation, as observed by Gaskell. Beyond  $pH$  5.9, however, the effect of acid was to cause contraction increasing up to a maximum with increasing acidity. Finally, beyond that maximum, as was the case too beyond a maximum contraction to alkalinity, relaxation set in.

The work of Hemingway and McDowall<sup>(6)</sup> showed that for perfusion experiments it was possible to maintain the tone of the capillaries by the previous administration of chloralose and repeated intravenous injections of alkali before the death of the animal, if after that the perfusion fluid was Ringer's solution at  $pH$  7.6. It seemed desirable to study with the same technique the effect of changes in the reaction of the perfused fluid and to determine if possible whether the vessels behaved in a way that was comparable with the results obtained by McSwiney and Newton, and at the same time see whether any new

which contain no phosphagen we have failed so far to observe any such synthetic process.

5. The resynthesis of phosphagen which occurs when a fatigued muscle is allowed to recover is very rapid if the surrounding atmosphere is rich in oxygen (one atmosphere or more). Under these conditions the restitution of phosphagen is far more rapid than the restitution of glycogen from lactic acid. Some resynthesis has been observed by Meyerhof in anaerobic conditions, but this is easily masked by the breakdown which is always occurring in the absence of oxygen, even in a resting muscle.

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The work of Hemingway and McDowall<sup>(6)</sup> showed that for perfusion experiments it was possible to maintain the tone of the capillaries by the previous administration of chloralose and repeated intravenous injections of alkali before the death of the animal, if after that the perfusion fluid was Ringer's solution at pH 7.6. It seemed desirable to study with the same technique the effect of changes in the reaction of the perfused fluid and to determine if possible whether the vessels behaved in a way that was comparable with the results obtained by McSwiney and Newton, and at the same time see whether any new

light could be thrown on the observations of Evans and Underhill, who also found conditions in which acid would cause contraction.

*Method.* The method adopted has already been described by McDowall and by Hemingway(7). It consists essentially of perfusing the vessels *in situ* from a reservoir of constant pressure and of recording the resistance of the vessels to the outflow by means of a side tube communicating with a piston recorder. The perfusion fluid was a modified Ringer's solution with a composition of NaCl 0.9 p.c., KCl 0.042 p.c.,  $\text{CaCl}_2$  0.024 p.c. heated to  $37^\circ$ , adjusted by the addition of  $\text{NaHCO}_3$  or NaOH to the pH required. Arrangements were made by which different perfusion fluids could be utilised, but they were not used because it was found that more convenient graphical records could be obtained by the method adopted by Hemingway and McDowall, in which the acid or alkali was injected through the tubing leading to the arterial cannula. In each instance 0.5 c.c. was injected, and from calculations made it was estimated that the fluid injected was diluted approximately ten times by the time it reached the elements on which it acted. Since the effects described are only relatively quantitative it was not necessary to determine more accurately the actual dilution. A small error is conceivably introduced by the fact that when the vessels are dilating and the perfusion fluid flowing more rapidly the injection becomes more diluted. Control experiments have, however, shown that this effect is negligible and, indeed, would tend to counteract the results actually obtained.

As a rule the vessels employed were those of the hind limbs of cats. Perfusion was begun either half an hour after decerebration or three or four hours after chloralisation and alkalisation. In the former case the animals are referred to as "unprepared" and are almost free from anæsthetic, in the latter they are referred to as "prepared." As was found by Hemingway and McDowall, in such animals lactic acid formation is delayed and the acid, as it is formed, is so neutralised that the reactions of blood vessels can be studied with a minimum of interference from the acid produced in the surviving tissue.

*Effects of dilute acid.* If the vessels of a "prepared" animal are perfused with Ringer's solution, having an alkalinity not less than pH 7.4, or preferably 7.6, the classical vaso dilator effect of acid first described by Gaskell is seen to occur with each of a series of injections. It is of brief duration, and at first rapidly recovered from, so that it can be repeated several times. Tracings illustrating the recovery have been published by Densham(8).

In "unprepared" animals, on the other hand, and when the alkalinity of the perfused fluid is less than  $pH$  7.4, the vessels do not recover from the dilatation caused by a sufficient dose of acid, the tone falls and a stage is reached in which subsequent injections give the reverse effect, constriction, with a dose which previously gave dilatation. Even in "prepared" animals perfused with fluid at  $pH$  7.6, a similar reversal may be obtained by giving still larger doses of acid.

The perfused vessels, then, not only of "unprepared" animals in which the accumulation of lactic acid has not been counteracted, but also, though only with larger doses of acid, or after several repeated doses, those of "prepared" animals, react to a sufficient amount of acid by constriction instead of dilatation.

A record of an experiment showing this is given in Fig. 1, where

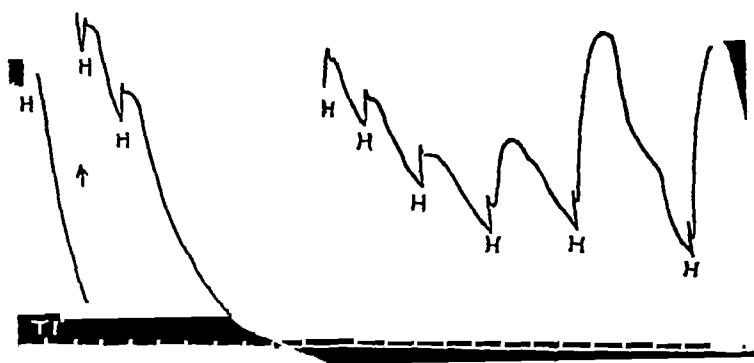


Fig. 1. The effect of acid (0.5 c.c.  $N/100$  lactic acid, see text) on alkaline tone. The first few injections of acid cause a typical dilatation, but gradually it is seen that there becomes developed a constriction before the dilatation. Eventually constriction is the only effect obtained. The injections were made at each  $H$ . A piece of tracing showing dilatation between 3 and 4 has been omitted.

vessels originally in a condition which may be called that of "alkaline tone" were, during perfusion, treated by a series of injections of 0.5 c.c.  $N/100$  lactic acid. The moments of injection are marked by the letter  $H$  and by an upstroke in the tracing, which is the constant mechanical effect of the injection of fluid whatever its nature into the system. The first six injections that are recorded caused simple dilatation (not allowed in this case to reach its maximum, in order to facilitate reproduction).



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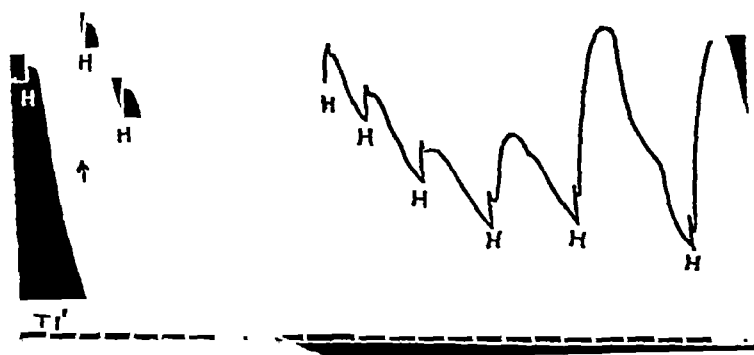


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In the experiment recorded in Fig. 2 a similar series of injections, but of stronger acid,  $N/10$ , were given. Each is seen to have caused in the first instance a well-marked but transitory constriction. After each constriction the tracing returns to an equilibrium position, descends, that is to say, and becomes horizontal before the next injection, so as to show what the effect on the level of tone has been. Thus it will be observed that the first dose of acid recorded causes a constriction followed by considerable loss of tone; the constrictions caused by the second and later doses are followed by gradual recovery of this lost tone, so that after the fifth, the process continuing, the tone rises to a level higher than before the first dose. An "acid tone" is being substituted for the lost "alkaline tone" and the second arrow marks the turning point.

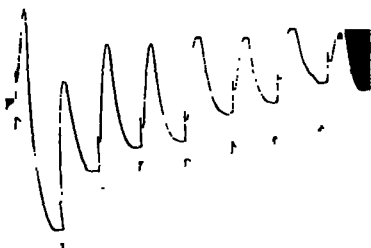


Fig. 2. The effect of a stronger dose (0.5 c.c.  $N/10$  lactic acid (see text)) than in Fig. 1. The effect of the first dose is a marked rise, followed by a dilatation. Subsequent doses give a constriction only.

*Effects of dilute alkali.* Hemingway has recorded the effect of a series of injections of alkali, showing how the same dose comes to have a much greater constrictor effect after several repetitions and how this augmented constrictor effect can be diminished by acid and restored again gradually by a series of injections of alkali.

An experiment is recorded in Fig. 3, in which a series of injections of alkali (0.5 c.c.  $N/100$  NaOH) were given during the perfusion of muscles originally in a state of "acid tone." The injections are marked by arrows; the first caused merely dilatation: at the second arrow a small constriction is seen to precede the dilatation, but this feature becomes more and more pronounced with the later doses. This constriction has been made a point of special attention both by myself and by Hemingway who, in describing the similar sensitisation by alkali, not only in perfused vessels but also in muscle from the uterus, showed clearly that this increased sensitivity was not dependent on changes in tone, although commonly associated with a reduction of the latter. The effect of alkali in increasing the action of drugs has long been known.

This experiment, therefore, indicated conditions under which alkali may cause dilatation; at first that and nothing else, later, though tone

continues to diminish with each dose, the immediate effect of each dose is constriction, and a constriction which, though it increases in amount

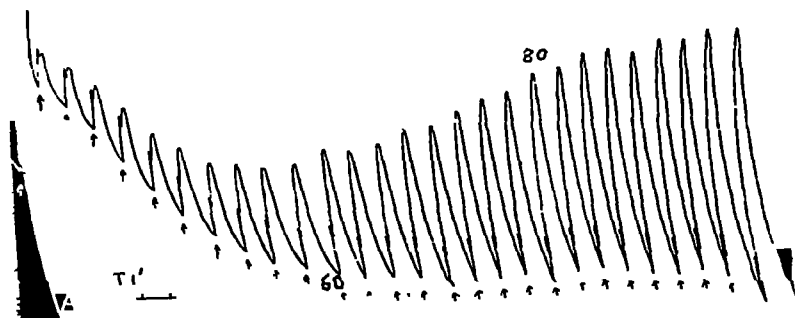


Fig. 3. The effect of alkali on "acid tone." At first the effect of the injection at each arrow of 0.5 c.c.  $N/100$  NaOH is a simple dilatation. With subsequent doses it is seen that a slight constriction effect appears and this becomes later increasingly prominent. The latter part of the tracing indicates that the increased sensitivity does not depend on an actual amount of contraction present.

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The conditions under which this dilatation occurs depend upon such accumulation of acid that the passage of alkaline fluid through the vessels, though it produces the immediate constriction regularly seen when there is normal, "alkaline," tone, nevertheless takes some time to wash the acid out of them and restore any degree of alkaline tone.

In some preparations the recovery from the alkaline constriction becomes less and less complete and a sustained alkaline tone eventually is produced. At the same time the immediate reaction to alkali becomes less (Fig. 4). Such a result is possibly explained by the acid of the tissues being eventually neutralised by the

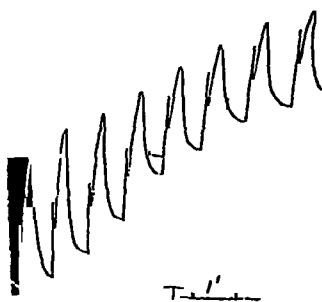


Fig. 4. Showing the increase of tone brought on by the injection of alkali (0.5 c.c.  $N/20$ ) with a perfusion fluid of pH 7.7, which of itself did not produce alkaline tone.

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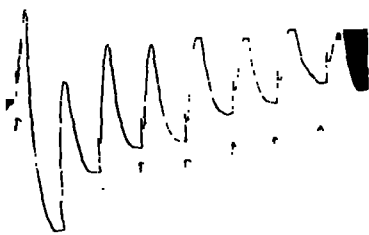


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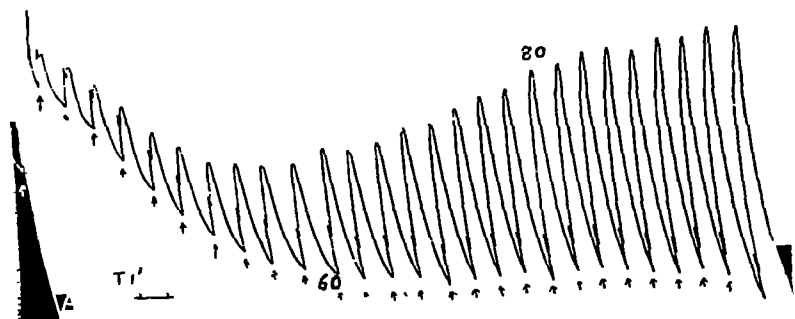


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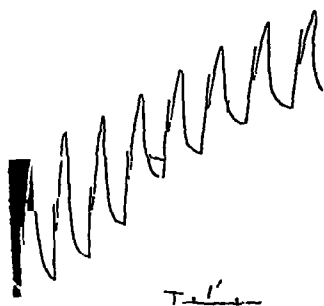


Fig. 4. Showing the increase of tone brought on by the injection of alkali (0.5 c.c.  $N/20$ ) with a perfusion fluid of pH 7.7, which of itself did not produce alkaline tone.

injection and the alkalinity of the perfusion fluid being then sufficient to maintain the alkaline tone.

It is clear, however, that the effect of dilute acids and also of dilute alkalis on the diameter of the vessels may be either to constrict or dilate them, according to the conditions that prevail at the time. When there is no accumulation of acid, alkalis constrict, acids dilate; when acid has accumulated, a point is reached at which acid constricts and may lead to a tonus that corresponds to nothing in the normal animal, and then alkalis are found to cause dilatation. On the approach of rigor mortis such a condition of "acid tone" appears to supervene naturally and in the ordinary course of things; for then the injection of alkali causes dilatation (see Fig. 5), as

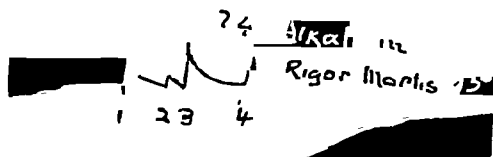


Fig. 5. The effect of alkali in rigor mortis. At 1, 2, 3 and 4, respectively, 0.25 c.c., 0.1 c.c., 0.5 c.c. and 0.5 c.c.  $N/100$  NaOH was injected. The first three doses were apparently without effect, but the fourth dose gave marked relaxation.

does perfusion with fluid at  $pH$  7.4, though not at  $pH$  7.1. When such "acid tone" is established the fluid issuing from the venous cannula has been found to be not more acid than  $pH$  6.9. If the injected fluid leaves all the capillaries at the same time, the reaction of the fluid leaving all the capillaries is the same and not more acid with some than with others, "acid tone" is induced by a very low degree of acidity. The seat of such "acid tone" appears to be the arterioles, since it is relaxed by acetyl choline and by nitrites, but not by histamine. If the capillaries were the seat of constriction in "acid tone" a small dose of histamine, insufficient to affect the arterioles, would be expected to dilate them and allow an easier escape of fluid, which is not found to be the case. It appears, therefore, that if capillaries are at any stage constricted by acid, since they are less effectively buffered than smooth muscle, the constrictor effect in them very rapidly gives place to a later phase of the action of the hydrogen ion, reached more slowly by the better buffered muscle, that of final, fatal, irreversible relaxation. Dr Dale tells me that he knows of no evidence for believing that capillaries ever

pass into a state of tone under the action of acid. The tendency to œdema in "acid tone" also points to the capillaries being dilated in this condition.

A general correspondence seems to be recognisable between these observations and those of McSwiney and Newton<sup>(5)</sup> on smooth muscle immersed in fluid at different values of  $pH$ , which they have summarised in a curve. At  $pH$  7.5 alkali caused constriction, acid relaxation: on the acid side of  $pH$  5.9 additions of acid caused contraction, alkali relaxation. The correspondence is qualitative only, the conditions of the experiments recorded here not allowing a quantitative correspondence to be detected: indeed, a quantitative correspondence should not probably be expected, because here smooth muscle is not the only tissue involved: there is no reason why capillaries should behave as smooth muscle and, as we have seen, there is reason for thinking they do not. The correspondence also extends to the extreme end on the acid side of the curve given by McSwiney and Newton; injections of acid, continued long enough, cause finally irreversible dilatation. The observation of the extreme effect of alkalies by the methods here used is rendered impracticable by complete closure of vessels before it can be reached.

From the above it is evident that in carrying out perfusion experiments it is necessary to pay special attention to the reaction of the perfusion fluid and of any substance which may be added to it. It may readily be demonstrated that the apparent passing off of the action of a drug on blood vessels is due not to a loss of sensitivity to the drug but to the production of acid by the dying tissues.

#### SUMMARY.

The effect of dilute acids and also of dilute alkalies on the diameter of the vessels may be either to constrict or dilate them, according to the conditions that prevail at the time. The conditions under which these results occur are described.

Part of the expense of this research was defrayed by the Government Grants Committee of the Royal Society.



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# THE ACTION OF INSULIN AND SUGAR ON THE RESPIRATORY QUOTIENT AND METABOLISM OF THE HEART-LUNG PREPARATION.

BY L. E. BAYLISS, E. A. MÜLLER<sup>1</sup>  
AND E. H. STARLING.

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University College, London.)*

## INTRODUCTION.

IN this paper we shall describe a new form of metabolism apparatus and the results obtained with it on the heart-lung preparation of the dog (Knowlton and Starling<sup>(1)</sup>). The respiratory quotient of this preparation has already been measured by Lovatt Evans<sup>(2)</sup>, but his figures are not altogether satisfactory for the following reasons. In the first place the preparation was acapnic, as indeed have been all those previously used for metabolism experiments, and in the second place the lungs were ventilated by a positive pressure, applied through the trachea, so that the lung capillaries were compressed and an abnormal work was thrown on the right heart. Moreover, there was a possibility of diffusion of gases through the lung walls and, in common with all previously described closed circuit metabolism measurements, the determination of the oxygen consumption rested on the assumption that the volume of the residual air in the lungs was constant; this assumption, we have reason to believe, is not always justifiable. We have, in fact, observed variations as large as 10-15 c.c. during the course of an experiment. We believe that our apparatus is free from these objections, and is capable of giving figures for the R.Q. with an uncertainty not greater than 3 p.c. to 4 p.c.

## METHOD.

The principle of our method consists in ventilating the lungs, by suction on the intra-pleural space, with a small, fixed quantity of air, in which the amounts of oxygen and CO<sub>2</sub> are kept constant by adding just so much of the one as the heart is using and removing just so much of the other as it is producing. This is rendered possible by measuring the percentage both of oxygen and CO<sub>2</sub> in this air continuously by a physical method.

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*Ventilating circuit.* As soon as the operations involved in making the heart-lung preparation were completed, the chest was closed, fixed in the position of maximum inspiration and made airtight, so that it provided a roomy enclosure for the heart and lungs.

This was done by eviscerating the animal, pulling the diaphragm downwards by threads attached to the rectum and filling the whole abdominal cavity with plaster of Paris, which was pressed, while setting, so as to expand the lower ribs and prevent them from collapsing. The skin incisions were made airtight after suturing by painting them with collodion and leakage through the mouth was prevented by tying a plug in the upper end of the trachea.

Negative ventilation was then applied to the pleural cavity in the following manner (Fig. 1). The two rotary valves,  $V_1$  and  $V_2$ , on the same

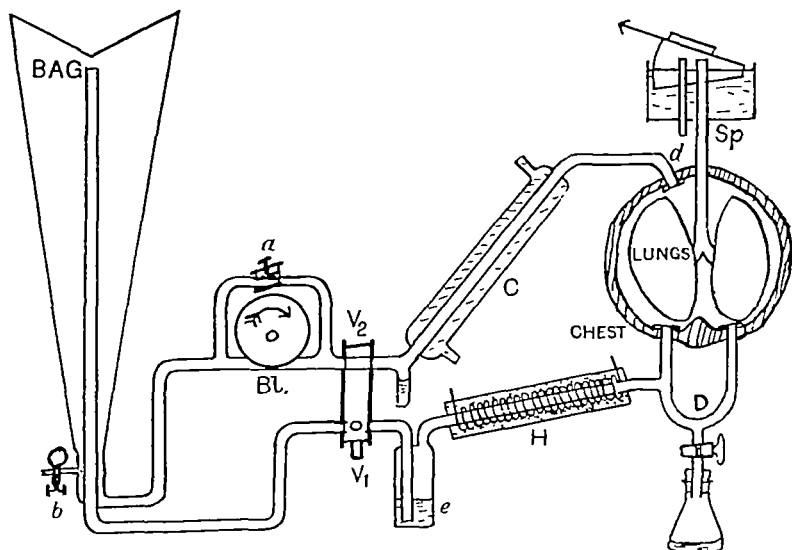


Fig. 1. Ventilating circuit.

shaft are opened and closed alternately about 12 times a minute. If  $V_2$  is open the rotary blower, *Bl.*, sucks air out of the chest through a suction tube (*d*) in the ventral wall, and the condenser *C*, and blows it into the Douglas bag. Since  $V_1$  is closed, a negative pressure is produced in the thoracic cavity and the lungs are expanded, drawing air in from the spirometer (*Sp*). The screw clamp, *a*, which controls a short-circuit across the blower, is adjusted to give a ventilation of about 150 c.c. per respiration. As soon as  $V_1$  opens and  $V_2$  shuts the lungs collapse and empty into the spirometer. The air thus aspirated into the pleural cavity from the bag passes through the heater *H*, which warms it to 35°, and then through

a T-piece connected with the blood-draining tubes in the dorsal wall. Complete collapse of the lungs is prevented by a wash-bottle, *c*, which maintains a negative pressure of 5 cm. of water in the chest.

For the experiments now in progress, we have modified somewhat the above arrangement of the valves. Instead of opening and closing rhythmically, they are operated electromagnetically in such a way that the lungs are expanded five times a minute to a predetermined volume or intra-thoracic pressure, whereupon they are immediately allowed to collapse. Contacts on a uniformly rotating shaft, and on either the spirometer or a manometer in the thorax, provide the stimuli for the motion of the valve. (This, incidentally, provides us with a good mechanical analogy of the Hering-Breuer reflex.)

Since in all our experiments we maintained the alveolar  $\text{CO}_2$  pressure at its physiological value of about 5 p.c. of an atmosphere, we also maintained the composition of the gas in this ventilating circuit at the same level, in order to prevent loss of gas by diffusion through the lung walls.

The magnitude of this loss by diffusion was estimated in the following manner. A normal preparation was run for about an hour and several consistent values of about 0.9 were obtained for the respiratory quotient; then the ventilating circuit was opened and atmospheric air allowed to circulate through the thoracic cavity. The r.q. fell immediately to 0.7, and rose again when 5 p.c.  $\text{CO}_2$  was once more circulated through the chest. We can calculate from this experiment that 1.3 c.c. of  $\text{CO}_2$  diffuse through the lung walls per minute under a pressure gradient of 5 p.c. of an atmosphere. This figure is in close agreement with that determined by measuring directly the rate of flow of  $\text{CO}_2$  through the lungs immediately after the heart had stopped. The lungs, in this experiment, were ventilated by a pump connected with a spirometer and a katharometer, and the closed chest was also connected with another spirometer and katharometer, so that by keeping the reading of the katharometer in the lung circuit constant and measuring the rate of change of the other, the rate of diffusion of  $\text{CO}_2$  through the lungs could be determined, since the volume of the thoracic system was approximately known.

It should be noted that this figure represents about 20 p.c. of the normal  $\text{CO}_2$  production, and allows us to calculate whether any significant loss was taking place during an experiment. We can also see from this that the changes in pleural pressure necessary for ventilation are not large enough to cause significant diffusion through the lungs.

The inclusion of a Douglas bag of 100 litres capacity in the ventilating circuit allowed small quantities of  $\text{CO}_2$  or oxygen to enter or leave the system without introducing serious errors, since they could only produce very small changes in the percentage composition. The volume of this bag was recorded continuously during the experiment, in order to check leaks, and the composition of the gas in it was measured at the beginning and end of each experiment; no significant changes in either were noted in any of the experiments recorded in this paper.

*Lung-spirometer system.* The volume of the air in this system was so small (about 500 c.c.), and the rate of ventilation so large, that the air in the spirometer was practically of the same composition as the air in the alveoli, so that we considered it sufficient for our purpose to deal only with the air in the spirometer. The composition of this we kept constant by means of the circuit shown in Fig. 2, adding just so much oxygen as the heart was using and removing just so much  $\text{CO}_2$  as it was producing.

The composition of the respiratory air was measured by means of two katharometers (Daynes(3)), the principle of which is that the temperature of a heated platinum wire depends upon the thermal conductivity of the gas surrounding it, so that, if the heating current remains constant, and convection is avoided, the resistance of the platinum wire will depend only on the composition of the gas. By this means, therefore, we were able to obtain continuous readings indicating the composition of the respiratory air.

The gas in the lungs consists of nitrogen, oxygen, carbon dioxide and water-vapour, all of which affect the readings of the katharometer. Since we desired to measure only the  $O_2$  and the  $CO_2$ , we had to supplement the katharometers with the appropriate absorption tubes.

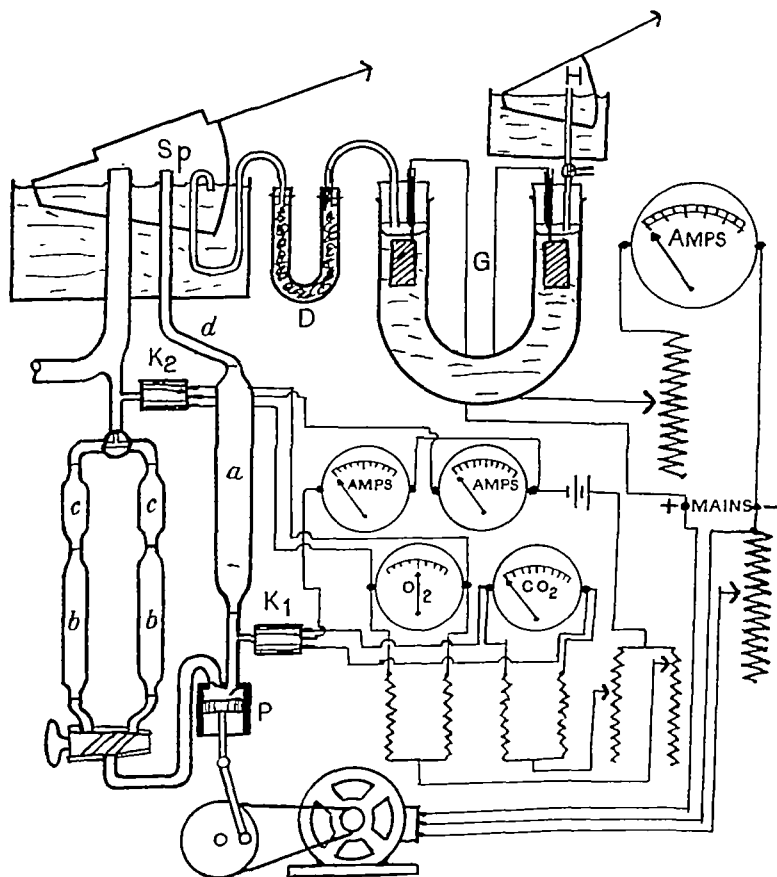


Fig. 2. Analysing circuit and controls.

*Analysing circuit.* Part of the air in the spirometer is driven around this circuit by the pump  $P$ . Leaving the spirometer  $Sp$ , it is dried in the calcium chloride tube  $a$ , and passes through the katharometer  $K_1$ . On the other side of the pump, the  $CO_2$  is absorbed in the soda-lime tubes  $b$ , and the air is re-dried in the  $CaCl_2$  tubes  $c$ , and re-enters the spirometer and lungs after passing through the katharometer  $K_2$ . Now at the point  $K_1$  the air consists only of nitrogen, oxygen and  $CO_2$ , and at the point  $K_2$  only of nitrogen and oxygen:  $K_2$ , therefore, will indicate the percentage of oxygen in the air at this point, and since the total amount of nitrogen in the system does not change, it will enable us to detect any change in the total amount of oxygen; this is independent, moreover, of any changes in lung volume, temperature or barometric pressure, in contradistinction to the determinations of the oxygen consumption by the Krogh(4) method. Again, so long as we keep the percentage of oxygen in the system at the value for which it was calibrated,  $K_1$  will allow us to detect any changes in the total amount of  $CO_2$ . Fortunately, the katharometers are much more sensitive to changes in the percentage of  $CO_2$  than they are to those of oxygen, so that  $K_1$  can be made much less sensitive than  $K_2$ , and hence is unaffected by the unavoidable slight variations in the oxygen percentage which are readily detected in  $K_2$ . Care was taken, of course, that the katharometers were not exposed to any interference from sudden temperature and pressure changes.

For the reasons given by Daynes(3), the katharometer is only suitable for exact measurements over a very limited range, so that, while invaluable for use in cases where a gas has to be kept at a constant composition, it is of little use when absolute measurements are required.

*The absorption and measurement of  $CO_2$ .* The soda-lime and  $CaCl_2$  tubes,  $bc$ , used for absorbing the  $CO_2$ , are weighed at intervals, one pair being weighed and put into position while the other is in action, so that by the arrangement of three-way taps, shown in Fig. 2, it can be substituted without stopping the flow of air. Since the percentage of  $CO_2$  in the air entering the analysing circuit is approximately constant, the amount of  $CO_2$  absorbed in unit time is proportional to the speed of flow through it, and this can be varied by varying the speed of the motor driving the pump; a scale on the speed-controlling resistance thus gives a semi-quantitative measure of the rate of  $CO_2$  absorption.

*The generation and measurement of oxygen.* Oxygen was generated and added continuously to the system through a water-seal in the spirometer at the appropriate rate by the method described by Rubner(5),

which consists essentially in the electrolysis of a strong solution of caustic soda. Ozone was decomposed by passing the gas through a tube containing manganese dioxide, and the rate of generation adjusted to the needs of the heart by altering the current in the generator by means of a series resistance; this current could be read on an ammeter and gave a rough idea of the rate of oxygen consumption of the heart at any minute. The volume of oxygen added was determined at suitable intervals by measuring in a calibrated spirometer (*H*) the volume of hydrogen evolved during the same interval; as soon as this was filled a stop-watch was started, and the volume of oxygen added to the system during the time taken to empty it in preparation for a second filling was calculated from the reading of this watch and of the ammeter during this time, the current in the generator being held constant.

This oxygen generating system was very carefully calibrated, and it was found that for the most usual current in the generator (1.5 amp.) the volume of oxygen actually evolved was 0.5 p.c. less than that calculated from the volume of hydrogen.

*Starting and running.* During the preliminary operations the preparation was ventilated with air containing the same proportion of  $\text{CO}_2$  as was subsequently used in the ventilating and lung-spirometer circuits, so that the blood and tissues were already in equilibrium with this at the beginning of the metabolism measurements. This air was analysed by the  $\text{CO}_2$  katharometer and the galvanometer deflection became the "standard  $\text{CO}_2$  value." As soon as negative ventilation was commenced and the system closed, one operator attended to the "artificial respiratory centre" and, by suitable adjustment of the controlling resistances, kept the deflections of the two katharometer galvanometers to the standard  $\text{CO}_2$  value and the standard  $\text{O}_2$  value (20.9 p.c.) respectively. The experiments were divided into "periods" which were determined by the time taken for the hydrogen gasometer to fill; this corresponded to a consumption of oxygen by the heart of about 80 c.c. and occupied usually 10 to 15 minutes. One period followed another immediately, the junction being determined by the passage of the index of the gasometer over a mark between the fiducial marks of calibration; in this way the emptying of the gasometer did not occur immediately before the end of each period. At this same moment the three-way taps at each end of the soda-lime tubes were swung over and a new pair of tubes brought into action, the galvanometers indicating the gas percentages were read, and the stop-watch corresponding to the previous period was stopped and a second one started.

*Corrections.* Corrections had to be applied to the values obtained for

the  $\text{CO}_2$  production and oxygen consumption during each period for the following reasons:

(a) Loss of  $\text{CO}_2$  by diffusion through the water-seal of the spirometer, the only place where it was found to be significant. This amounted to 0.1 c.c. per minute when there was 4 p.c. to 5 p.c. of  $\text{CO}_2$  in the system.

(b) Deviations of the gas percentages from the standard values. These were easily calculated from the galvanometer readings at the junctions of the periods and the volume of the system. The corrections were of the order of  $\pm 0.3$  c.c. of  $\text{CO}_2$  and  $\pm 0.5$  c.c. of oxygen. The deviations of the oxygen katharometer did not affect the  $\text{CO}_2$  katharometer unless they indicated a correction of more than 1.0 c.c. of oxygen. Changes in the total  $\text{CO}_2$  of the blood were usually negligible.

*Accuracy.* Unfortunately it has not proved possible, so far, to calibrate the whole apparatus by means of the alcohol flame, since a flame small enough would not keep alight in 5 p.c.  $\text{CO}_2$  in air. It is necessary, therefore, to estimate separately each source of error. These are (a) the lag in the response of the katharometers to changes in the composition of the air, (b) the admission of oxygen from the generator through the water-seal in the spirometer, since it is liable to be admitted in groups of bubbles, sometimes as much as 1 c.c. at a time, (c) changes in the standard values of the galvanometers owing to the drying tubes passing traces of water-vapour to the katharometers, and (d) leakage in the thoracic circulation causing a change in the  $\text{CO}_2$  percentage of the air outside the lungs.

Of these, the first two are considerably reduced in magnitude by taking the average values of the  $\text{O}_2$  consumption and  $\text{CO}_2$  production during a large number of periods, since it can easily be seen that a positive error in the first period, for instance, would lead to an equal negative error in the second, so that the error of the average would be reduced in simple proportion to the number of observations and not to the square root as is the case with purely random errors. The magnitude of the second two can be determined by taking the readings of the galvanometers with atmospheric air in the system, and by measuring the  $\text{CO}_2$  percentage in the thoracic circulation, respectively, at the end of the experiment.

In addition to these, we have to include the random errors incident to the actual measurement of the  $\text{CO}_2$  and  $\text{O}_2$ , and by adding together the estimates of the magnitudes of these six individual sources of error, we get the uncertainty of our R.Q. measurements. This is found to be  $\pm 4$  p.c., of which  $\pm 1.5$  p.c. is due to the measurement of the  $\text{CO}_2$  production, and  $\pm 2.5$  p.c. to that of the  $\text{O}_2$  consumption; in practice, our actual measurements indicated a somewhat greater accuracy.



*The heart-lung preparation and blood circuit.* A closed circuit preparation was used of the type already described by Starling and Visscher<sup>(6)</sup>, but a few alterations in technique were necessitated by the adoption of negative ventilation and the desire to keep the total volume of blood in circulation as small as possible (300 to 500 c.c.). The blood of the dog, from which the preparation was made, was accordingly adequate by itself, and we were able to reduce the volume of "dead" gas in the blood to a minimum, but were forced to take extra precautions against loss of blood by leakage.

Six ligatures were tied around the descending aorta, so as to block anastomoses and leakage into the intercostal vessels, the œsophagus was tied and the last ligature was made to include the trachea, a long canula being used to prevent its being constricted. All the blood that leaked into the chest was drained off and returned into the circulation, but experiments in which large quantities had to be so returned were regarded with suspicion, since this blood might not always be in true gaseous equilibrium with the respiratory air.

Clotting was prevented by the addition of heparin, the total amount of saline added being reduced to the amount necessary to dissolve the heparin, since we found that excessive quantities were liable to cause early lung œdema.

The blood-sugar was determined by the Hagedorn-Jensen method<sup>1</sup> and the alkali reserve was determined at the beginning and end of each experiment, and occasionally in the middle, by analysing samples with a Van Slyke manometric apparatus. No significant changes were observed, the figures for one experiment being 38.6 vols. p.c. CO<sub>2</sub> at the beginning of the experiment and 38.4 vols. p.c. two hours later.

The venous pressure was used to indicate the heart volume, since a cardiometer is impracticable with a closed chest and negative ventilation; this is justifiable since the output and the arterial pressure were maintained constant (at 500 c.c. per min. and 100 mm. Hg respectively). The venous pressure will depend to some extent, of course, on the intrathoracic pressure, but since, in our experiments, the lungs were always allowed to collapse to a constant pressure, this factor is insignificant provided that the venous pressure is read at the end of expiration.

The output of the heart was measured in a simple form of stromuhr, in which, by turning a three-way tap, the blood could be collected in a graduated vessel instead of the venous reservoir, and returned to the circulation afterwards. In order to prevent the output from varying during the course of an experiment, it is necessary that the head of blood in the venous reservoir should be independent of volume within it, and this was accomplished by connecting the water-jacket surrounding the rubber sleeve, which served as venous reservoir, with a vessel suspended

<sup>1</sup> We are indebted to Dr Isolde T. Zeckwer for these determinations.

by means of a long rubber tube. As the sleeve fills with blood it displaces water into the suspended vessel which consequently becomes heavier, stretches the rubber tube and sinks. By adjusting the length and diameter of this tube, it is possible to make the vessel sink just so much that the water in it remains at the same level in space for all amounts of blood in the venous reservoir, provided, of course, that the suspended vessel has a uniform cross-section throughout its length. This method allowed us, also, to observe the amount of blood in the reservoir, which indicated, in a rough way, rapid changes in the volume of the heart. The temperature of the water in the jacket was kept constant by means of a regulator of the usual pattern.

### RESULTS.

Starling and Visscher(6) have shown that the energy requirements of the heart for a given stroke volume increase with an increase in the diastolic volume so that the efficiency decreases; this has been confirmed more rigidly by Hemingway and Fee(7). Now in the heart-lung preparation the heart slowly and continually dilates although, according to Starling, under physiological conditions it always works at the smallest possible volume and with a maximum efficiency, and Visscher and Muller(8) have shown that this dilatation can be abolished by the addition of insulin.

We have been able to confirm these observations, as will be seen in Fig. 3. Owing to the fact that the operation and the starting up of the apparatus took about three hours, the preparation at the beginning of the metabolism measurements was already beginning to lose efficiency, as is shown by the rising metabolism and venous pressure, which, it must be remembered, is determined only by the heart volume, the output and arterial pressure being kept constant. Although, up to the present, we have been unable to determine exactly the effect on the R.Q. of varying the efficiency of the heart, there are definite indications that constant values will not be obtained when the heart volume is varying rapidly; further work on this question is in hand. It is impossible, therefore, to determine the R.Q. of the heart under normal conditions from the data presented in Fig. 3, but as can be seen in this, and more clearly in Fig. 4, it was always possible to bring down the venous pressure and the metabolism by the addition of insulin. This effect was only transitory, however, unless glucose was also given, either before or after the insulin, when it lasted for an hour or more. Neither had any action on a heart already working with a good efficiency. The R.Q. is initially 0.9 and

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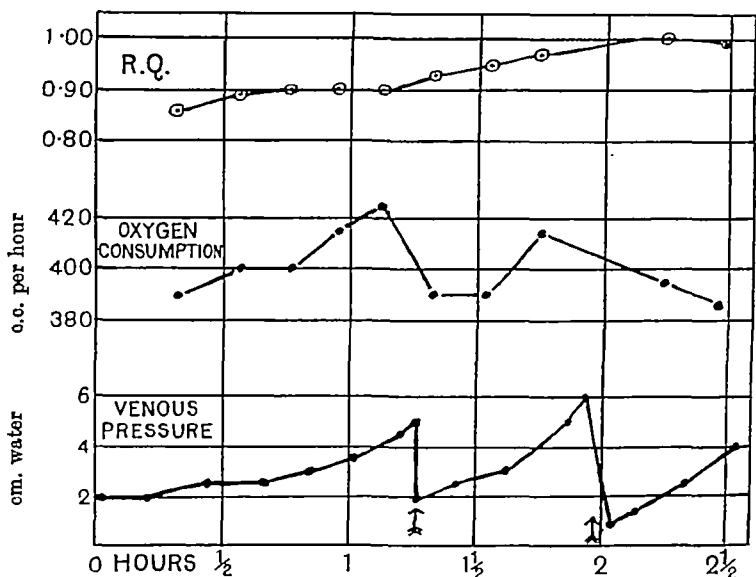


Fig. 3. From below upwards, venous pressure (from arbitrary zero), oxygen consumption and R.Q. At the first arrow, 1 gm. glucose was added and at the second 10 units insulin. Output 500 c.c. per min., arterial pressure 103-4 mm. Hg, heart weight 88 gm., alveolar  $\text{CO}_2$  3.75 p.c.

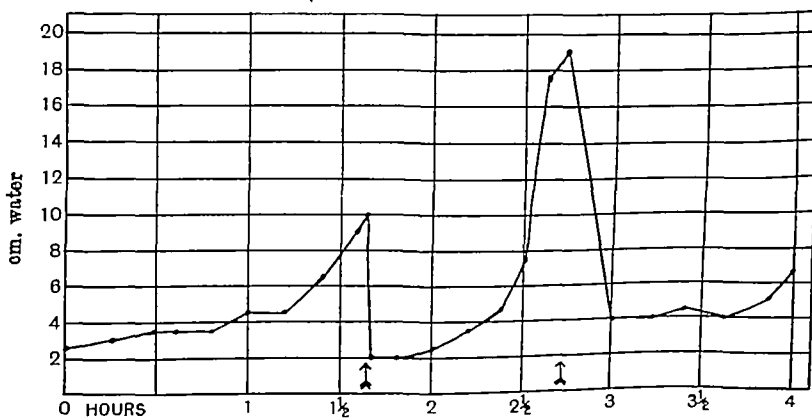


Fig. 4. Venous pressure (from arbitrary zero). At the first arrow, 10 units insulin were added and at the second 10 units insulin and 1 gm. glucose.

subsequently rises, although much reliance cannot be placed on these figures.

By continually injecting insulin and sugar we have been able to maintain the heart in good condition for as long as six or seven hours; Fig. 5 presents the results of such an experiment, 0.12 c.c. of a solution

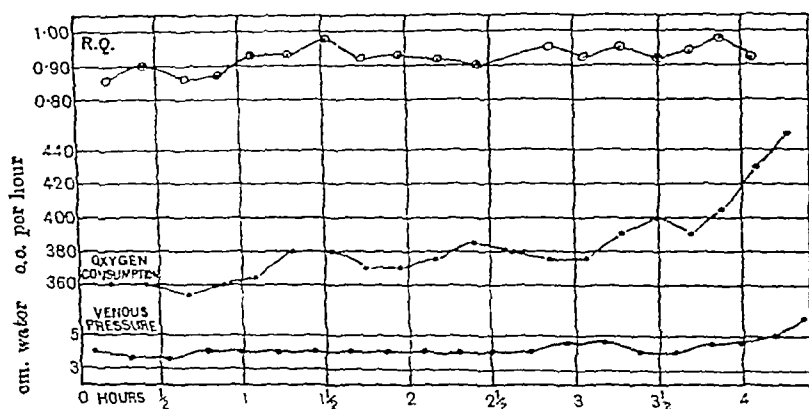


Fig. 5. From below upwards, venous pressure (from arbitrary zero), oxygen consumption and R.Q. Continuous sugar and insulin injection. Output 550 c.c. per min., arterial pressure 100 mm. Hg, heart weight 60 gm., alveolar  $\text{CO}_2$  3.9 p.c.

containing 0.058 gm. glucose and 0.38 unit insulin being added to the blood every four minutes, this being about 50 p.c. more sugar than the heart is using and doubtless an enormous excess of insulin, although no attempts have yet been made to investigate this<sup>1</sup>. It will be seen that the average R.Q. for the whole experiment is  $0.925 \pm 0.025$  (mean error).

Since the heart volume can be reduced by glucose alone as well as by insulin, we attempted in three experiments to keep the heart in a good state by the addition of glucose alone, 1 gm. being given as soon as the venous pressure began to rise. The results of one such experiment is shown in Fig. 6, and it is seen that the concentration of glucose in the blood rises steadily with the time until after two hours it has reached 2.26 p.c. The points on the blood-sugar time curve marked as "calculated" were obtained from the known amounts of sugar added to the blood, allowance being made for the quantity used by the heart and lost in the blood by leakage. If, now, insulin is given, the heart will continue in good condition for a considerable period without any further addition

<sup>1</sup> More recent experiments have shown that injections containing 1/25 of this amount of insulin are adequate.

of glucose, the blood-sugar concentration falling at the same time at a rate very much greater than would be accounted for by the sugar

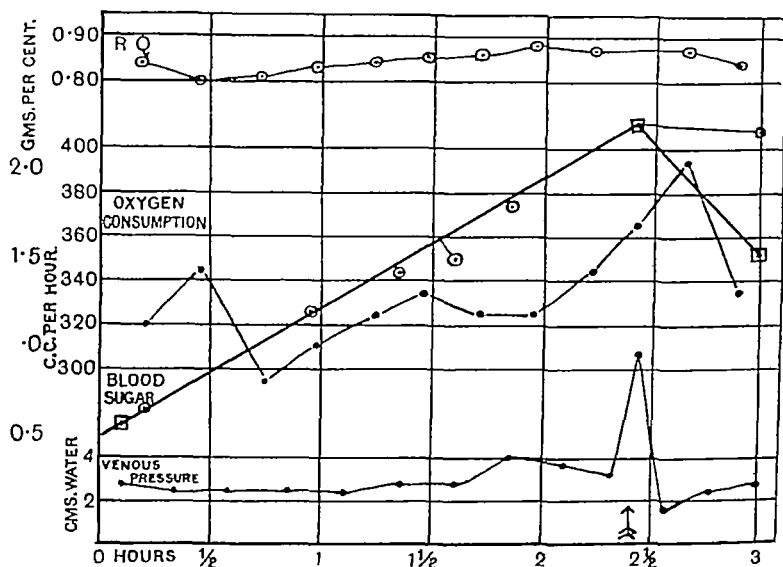


Fig. 6. From below upwards, venous pressure (from arbitrary zero), blood-sugar concentration, oxygen consumption and R.Q. Calculated blood-sugars in circles, observed in squares. Output 500 c.c. per min., arterial pressure 102–3 mm. Hg, heart weight 65 grm., alveolar  $\text{CO}_2$  5.0 p.c. At the arrow, 10 units insulin were added. Lung oedema followed and the output dropped.

consumption of the heart. In the two experiments in which this phenomenon was observed, the ratio  $\frac{\text{sugar disappearing}}{\text{sugar burnt}}$  was 19 and 9 respectively. Three experiments have been performed without addition of insulin and the average values for the R.Q. were

$$0.855 \pm 0.065, \quad 0.840 \pm 0.020, \quad 0.910 \pm 0.025,$$

respectively, somewhat smaller than those obtained in the presence of insulin, but it is doubtful if the difference is significant.

In a preparation of this kind it ought to be possible to determine the effect of insulin on the R.Q., and the results of an attempt at this are shown in Fig. 7. In every experiment of this kind that we have performed, however, the addition of insulin has caused a definite reduction in the venous pressure, so that the heart was not in exactly the same condition before and after the insulin, but neglecting this, a first analysis would seem to indicate that the addition of insulin causes a small rise in the R.Q., the average R.Q. being  $0.890 \pm 0.025$  before insulin and

0.935  $\pm$  0.025 after insulin. Such a conclusion is shown to be erroneous. however, when the values of the R.Q. obtained in this experiment are

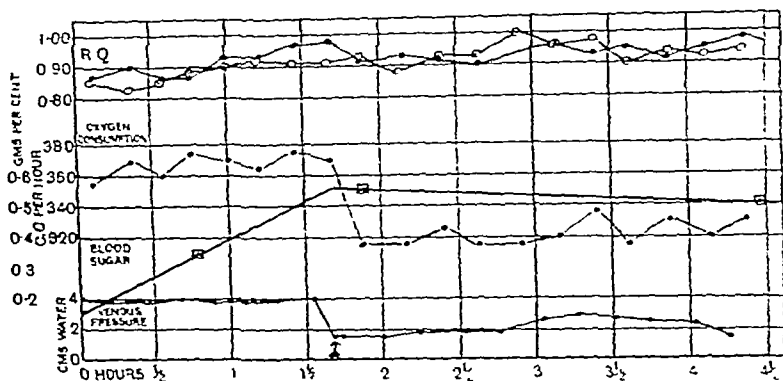


Fig. 7. From below upwards, venous pressure (from arbitrary zero), blood sugar concentration, oxygen consumption and R.Q. In solid dots R.Q. values from Fig. 5. At the arrow, 10 units insulin were added. Output 500 c.c. per min., arterial pressure 101 mm. Hg, heart weight 80 grm., alveolar  $\text{CO}_2$  4.8 p.c.

compared with those obtained in the experiment in which insulin was given continuously, which are plotted as solid dots in Fig. 7. The two sets of values superpose almost completely, and the cause of the rise in the R.Q. must be sought elsewhere.

Some experiments have also been made on hearts from de-pancreatised dogs, with a view to determining whether such hearts had any different type of metabolism from those of normal animals. The experiments were in all cases very short, however, owing to heart failure, and were performed before the apparatus was really satisfactory, so that no great reliance should be placed on the results. Nevertheless, in all cases the R.Q. lay around 0.8, and there seemed to be a rise after the addition of insulin, although this is probably not significant. It is interesting to note that the effect of insulin on the venous pressure of these preparations was exactly the same as that in normal preparations. It seems probable, moreover, that these hearts could have been kept in good condition for longer periods by increasing the sugar concentration of the blood in the same way as could the normal hearts in the absence of insulin.

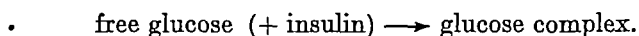
When we refer to insulin in this paper, we mean the commercial preparation (B.D.H.) that we happened to use, and we have no conclusive evidence that the substance that acts as a heart tonic is the same as that which causes a fall in the blood-sugar, although there is considerable circumstantial evidence for it.



## DISCUSSION.

Our experiments show definitely that the R.Q. of the heart-lung preparation is not greater than 0.95 and is probably less. It is possible that the R.Q. of that part of the total metabolism which is responsible for the mechanical work of the heart is unity and that there is also a "trophic" metabolism with a low R.Q. in addition; it must not be forgotten, in this connection, that the metabolism of the lungs is included in all our figures. Since the addition of insulin or glucose to a heart not previously treated with either, increases its efficiency, the question whether variations in the quantities of these substances present affect the R.Q. is insoluble at present.

In the absence of insulin enormous concentrations of glucose are needed in the blood in order to permit the heart to function properly, and subsequent addition of insulin causes a rapid disappearance of much of this glucose. This disappearance, however, only takes place when the sugar concentration is initially greater than 0.5 p.c., so that the observations of Plattner<sup>(9)</sup> are confirmed. We have definitely excluded the possibility that this sugar is caused to disappear by combustion, thereby confirming the observations of Burn and Dale<sup>(10)</sup>, and we suggest that our results might be accounted for by postulating a reaction between free glucose and a substance that we might term "stored glucose," or better "glucose complex," in which insulin plays a part, so



We are not in a position to state whether the insulin is a reactant or a catalyst, but if it is a catalyst, it must be removed from the system by a secondary reaction at a uniform rate. In the normal animal the main reaction is proceeding from left to right and the glucose complex so formed constitutes the actual material used by the heart muscle cells to provide the energy for their contraction; any excess of production over consumption is laid down in the stores. Let us now consider a few applications of the Law of Mass Action to this hypothetical system. (1) If the concentrations of insulin and glucose in the blood are small, the velocity of formation of the glucose complex is small, and may be less than the rate of consumption, so that the stores are depleted and the heart begins to fail. (2) By giving insulin or glucose, the reaction is speeded up, the stores are refilled and the heart recovers, but only for a short time, since the concentration of the other component is rapidly diminishing; conversely, it is only to be expected that addition of these substances to a

heart with stores already filled would have no action. (3) If glucose only is given, it is clear that the concentration required to maintain a given rate of formation of the glucose complex (*e.g.* that equal to the rate of its consumption) will increase with time as the amount of insulin present decreases, and if the rate of insulin disappearance is constant, the glucose concentration in the blood should be a linear function of the time. (4) If insulin is given when the blood-sugar concentration is high, the rate of formation of the complex will exceed the rate of consumption, and it will be laid down in the stores without any increase in the metabolism. Much further work is obviously needed, however, before these suggestions can be regarded as anything more than purely hypothetical.

## SUMMARY.

(1) A new method of determining the R.Q. and metabolism of the heart-lung preparation is described, in which acapnia, positive ventilation and the participation of factors involving the lung volume, barometric pressure and atmospheric temperature, are avoided.

(2) The R.Q. of the heart-lung preparation is not greater than 0.95.

(3) Introduction of insulin and glucose, the former in relatively large amounts will maintain the preparation in good condition for long periods. A large concentration of the latter will compensate to a certain extent for a deficiency of the former.

(4) Insulin and glucose have no specific effect on the metabolism, since they only change it if the heart volume changes at the same time.

A great part of the expenses of this research was borne by a grant to the late Prof. E. H. Starling by the Foulerton Research Committee of the Royal Society. It is greatly to be regretted that, although the greater portion of the work was done under his personal supervision, it was not completed in time for him to be able to take part in the presentation of its results.

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# THE ACTION OF INSULIN ON THE PERFUSED MAMMALIAN LIVER.

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Hampstead, London, N.W. 3.)*

## INTRODUCTION.

THE striking reappearance of glycogen in the liver of the diabetic animal after insulin treatment has led to the justifiable assumption that insulin plays an important part in promoting glycogen storage in the liver. Many attempts have been made to prove the truth of this assumption on the normal animal, but the results of these experiments have not been in agreement. Thus, in either starved or fed normal animals, insulin alone was observed to cause a loss of glycogen from the liver, while when it was given in conjunction with glucose, the results were variable. Macleod(1), for instance, observed a loss of glycogen, Babkin(2), no change, and Cori(3) and Collazo(4), a storage.

This result on the whole animal has not been accepted as convincing evidence against the theory, since the direct action of insulin on the liver might well be masked by a complicated interaction between the various organs. One or two investigators, therefore, have attempted to decide the question by experiments on the isolated liver. Of these, we will mention only the experiments of Bornstein and Griesbach(5), and of von Issekutz(6). (A comprehensive review of the literature is given by Grevenstuck and Laqueur(7).) The former, working on dog's liver, observed that the progressive breakdown of glycogen which they normally obtained was unaffected by insulin, but that the large increase in glycogenolysis caused by adrenaline was abolished by insulin. Von Issekutz(6), in a recent paper, has also described the abolition of the adrenaline effect by insulin, and attributes this to diminution of the increased acid production which he observed after adrenaline alone. Insulin alone did not affect the glycogenolysis, but diminished the acid production which he regularly observed in his experiments. The results of these observers cannot be accepted as convincing, since the fact that they were unable to stop the progressive breakdown of glycogen in their

experiments, whether or not insulin was given, indicates that the liver was not in a strictly physiological condition. For, if the liver had been in a condition corresponding to that in the living animal, a storage of glycogen should have been observed at some stage in the experiment, perhaps only after the administration of insulin, on the assumption that insulin is necessary to the glycogen storage.

The present paper also deals with the effect of insulin on the carbohydrate metabolism in the isolated mammalian liver perfused with a technique somewhat different from that employed by previous workers.

### METHODS.

During the course of the investigation we have had to make many alterations in the technique, but only that finally adopted will be described in full. In the first place we may draw attention to the various particulars in which our technique differs from that employed by others. These are:

(1) The perfusion was carried out through both the hepatic artery and the portal vein, under pressures of 100 mm. and 15 mm. of mercury respectively. This was facilitated by the use of the Dale-Schuster pump recently described (8) modified to the extent that the portal pump, instead of supplying the blood straight to the portal vein, delivered it first into a reservoir, from which it was fed by gravity into the portal vein. This was done in order to remove any pulsation from the portal circulation.

In the earlier experiments the hepatic artery was supplied with arterial blood, and the portal vein with venous blood, but later, in order to simplify the apparatus, arterial blood was perfused through the portal vein as well.

(2) The whole preparation and isolation of the liver were carried out without interruption of the arterial circulation, and, therefore, of the oxygen supply to the liver.

While the heart was still supplying the liver with blood through the hepatic artery a temporary cannula was inserted in the pancreaticoduodenal artery and the artificial perfusion started backwards to the liver while it was still *in situ* (Fig. 1). This artificial perfusion being established, the blood supply from the heart by way of the main hepatic artery was stopped, and a second cannula inserted into this vessel. After the isolation of the liver this cannula was used for the perfusion proper. In the later experiments, even the blood supply to the portal vein was interrupted only for the short time necessary for the insertion of a cannula, and the setting up of the artificial perfusion.

The aeration of the blood was carried out by including isolated lungs in the circuit. In one or two of the first experiments a mechanical

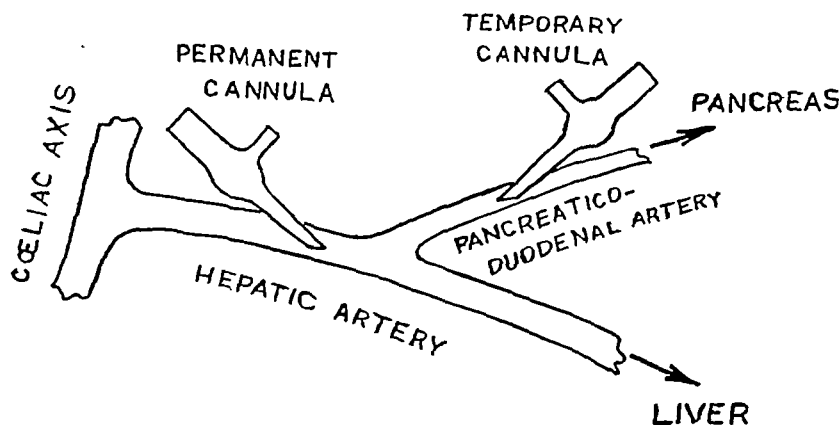


Fig. 1.

oxygenator was used, but it was found that the freshly defibrinated blood exerted a strong vaso-constrictor action on the arteries of the liver, similar to that previously observed by Starling on the kidney vessels, and in this Institute on the vessels of the isolated hind leg.

It was first pointed out by Bodo and Gremels(9) that this vaso-constrictor action of defibrinated blood can be removed by circulating it through the heart-lung preparation for 20-30 minutes. The lungs appeared to be the most likely seat of this change, and indeed, experience in this Institute and elsewhere has shown that the lungs are responsible for the removal of this vaso-constrictor effect. By substituting the isolated lungs for the mechanical oxygenator in our experiments, and circulating the blood through them for about half an hour before the perfusion was started, we avoided trouble from vaso-constriction, as indicated by absence of rise in resistance, measured by a manometer connected to the hepatic artery cannula.

It is important that the blood should be well oxygenated throughout the whole of the experiment. In cases where the oxygenation failed in the later stages, on account of cedema of the lungs, a breakdown of glycogen was observed. In order to avoid this a fresh pair of lungs was substituted as soon as any sign of deficient oxygenation appeared. This presented no difficulty since at least two dogs were used in each experiment.

The temperature of the blood supply to the liver is important. It was found in our experiments that the best results were obtained when the

temperature of the blood entering the liver was between  $38.5^{\circ}$  and  $39.0^{\circ}$  C. High or low temperatures caused a breakdown of glycogen.

A 25 p.c. solution of glucose was added to the blood in sufficient quantity to raise the blood sugar level to between 1 and 2 p.c. When this was omitted, no glycogen storage was observed.

As is well known the liver loses a large part of its glycogen under ether anæsthesia. and in corroboration of this we have found that the liver contains very little glycogen after deep anæsthesia with ether alone. In order to reduce to a minimum the amount of ether required to produce complete anæsthesia. we therefore administered beforehand a large dose of morphia.

All the experiments were performed on dogs, of which two or three are required in order to obtain sufficient blood to continue the perfusion for hours. The dog from which the liver was removed provided but little of the blood used for the perfusion.

After one or two dogs had been bled out under deep ether anæsthesia, the lungs of one were prepared by inserting cannulæ in the pulmonary artery and left auricle respectively, and by tying a ligature round the atrio-ventricular ring. The lungs were then isolated, connected with the apparatus so that they lay on the plate ( $P_2$ ) which was heated from below by a spiral of lead tubing through which warm water flowed, and the trachea was attached to a Schuster respiration pump so as to produce a positive ventilation.

The lungs were then perfused with defibrinated blood, warmed by passage through the jacketed tube ( $J$ ), the oxygenated blood from the lungs being collected in the reservoir ( $R_2$ ), from which it is provisionally returned to the reservoir ( $R_1$ ), through the hepatic side ( $HP$ ) of the Dale-Schuster pump.

Meanwhile, the remaining dog was being prepared for isolation of the liver, the operation taking at least half an hour, so that the blood had already been circulating through the lungs for this period, before the perfusion of the liver was started. The dog, which one hour previously had received subcutaneously 20 mgr. of morphine sulphate per kilo body weight, was completely anæsthetised with a small amount of ether, and the chest and abdomen opened under artificial respiration, and a cannula introduced into the bile duct. After injecting heparin (1 mgr. per 5 c.c. of blood) into the external jugular vein in order to prevent clotting, the pancreatico-duodenal artery was prepared, the temporary cannula inserted, connected to the arterial side ( $HP$ ) of the pump and the perfusion started with the blood to which, as previously noted, glucose had been

added. The hepatic artery was immediately clamped close to the coeliac axis, and the arterial perfusion pressure, as indicated by a manometer attached to the side-tube (*HM*), adjusted to about 100 mm. of mercury by regulating the stroke of the hepatic pump. Thus the natural arterial supply to the liver was immediately replaced by the artificial perfusion of blood at 100 mm. pressure and a temperature of 38.5°–39° C.

At the same time, the animal was bled from the carotid artery, and the inferior vena cava opened in the chest, the issuing blood being collected with clean cotton-wool, filtered, and returned to the reservoir (*R*<sub>1</sub>) for oxygenation by the lungs. The outflow from this reservoir was adjusted by means of a screw clip, in order to prevent air from entering the lungs.

The portal vein was next prepared, a cannula introduced and, in our later experiments, connected immediately with the portal pump (*PP*) so that the portal circulation also was established *in situ*. Finally, the second cannula was tied into the main hepatic artery, and the liver completely isolated and transferred to the plate (*P*<sub>1</sub>) without stopping the perfusion. This plate was warmed by a coiled tube similar to that used for the lungs and was provided with a hole in the centre, covered with a muslin filter through which the blood issuing from the vena cava (cut quite close to the liver) drained directly into the reservoir (*R*<sub>1</sub>).

The second cannula was now connected to the pump, and the pancreatico-duodenal artery tied off and its cannula removed.

The scheme of perfusion is therefore as follows (Fig. 2): The blood issuing from the liver is collected in the liver reservoir (*R*<sub>1</sub>), from which it passes to the lungs through the jacketed tube (*J*). Issuing from the lungs, it is drawn from the lungs-reservoir (*R*<sub>2</sub>) by the Dale-Schuster pump, one side of which delivers straight to the hepatic artery (*HA*) and the other into the portal reservoir (*R*<sub>3</sub>), from which the blood passes by gravity into the portal vein (*PV*), under a hydrostatic pressure of about 15 mm. of mercury, measured by the manometer (*PM*). The water for warming the plates (*P*<sub>1</sub>) and (*P*<sub>2</sub>) and the jacket (*J*) was heated by passing through the flask (*F*), heated by a bunsen burner, while the bath surrounding the Dale-Schuster pump was maintained at 40°–41° C. The temperature of the blood entering the liver was measured by thermometers in the cannulae. Samples of liver for the estimation of glycogen were removed simply by cutting off a piece weighing about 5 gm. from each of two lobes. It was unnecessary to tie off the remaining part of the lobes, the blood issuing from the cut surfaces being allowed simply to join the main outflow draining into the reservoir (*R*<sub>1</sub>). It was only necessary to increase

slightly the stroke of the hepatic pump so as to maintain the pressure of 100 mm.

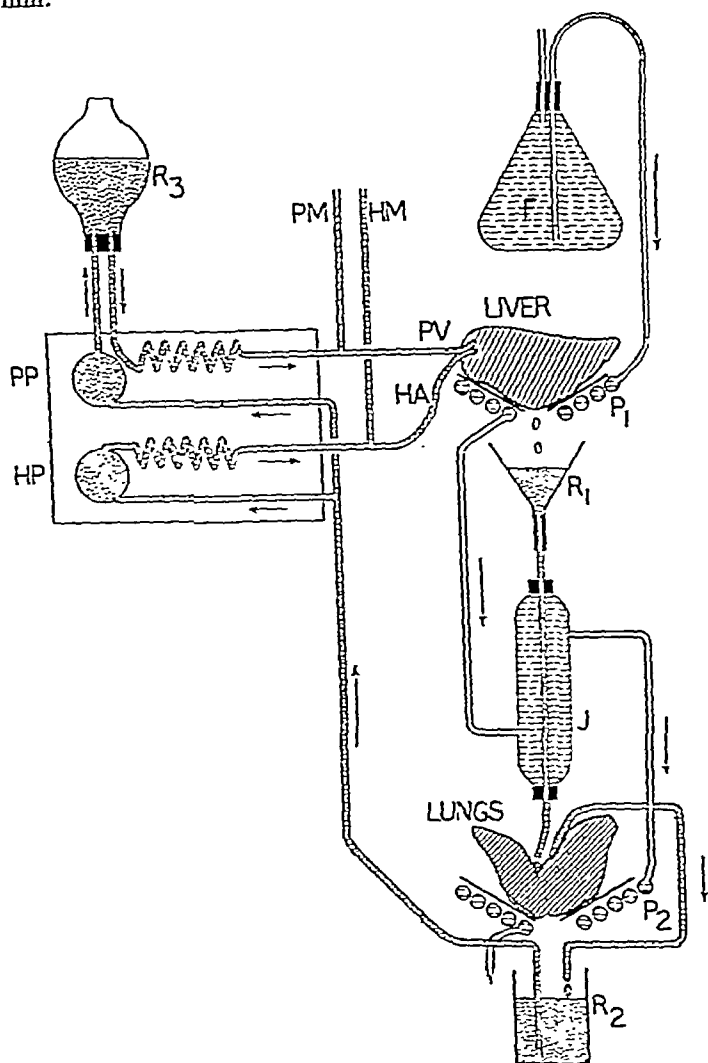


Fig. 2.

In this way we were enabled to take further samples from the same two lobes at half-hourly intervals during the whole of the experiment. This was important since, in confirmation of Macleod (10), we found that after ether anæsthesia the different lobes contained varying amounts of



glycogen. In the majority of our experiments the first samples were taken immediately after the complete isolation of the liver, but in some of the later ones samples were also taken from the liver *in situ* at the time of starting the artificial perfusion. The glycogen was estimated by the modified Pflüger method (11).

Samples of blood were withdrawn, usually from the portal reservoir,

### Experiments without insulin.

Exp. 1. Liver weighing 200 grm. perfused with 1400 c.c. blood containing 25 grm. glucose.

		Hepatic artery			Portal vein
Pressure ...		100 mm.			15 mm.
Temperature ...		38.6° C.			38.4° C.
		Percentage liver glycogen			
	Per-centage blood sugar	I lobe	II lobe	III lobe	IV lobe
Time					
2.40	2.22	1.96	1.60	—	—
					Samples taken <i>in situ</i> at time of starting arterial perfusion
3.15	1.86	.53	.75	—	—
					Isolation of liver complete
3.45	1.86	.61	.58	—	—
4.15	—	.78	.72	.56	.57
					Samples taken from two fresh lobes
4.45	1.84	—	—	.71	.71
5.15	1.81	—	—	.70	.64

Exp. 2. Liver weighing 234 grm. perfused with 1600 c.c. blood containing 7.5 grm. glucose.

				Hepatic artery		Portal vein
		Pressure	...	...	105 mm.	15 mm.
		Temperature	...	...	39.0° C.	38.0° C.
		Percentage liver glycogen				
	Per-centage blood sugar	I lobe	II lobe	III lobe	IV lobe	
Time						
2.25	.75	1.60	1.06	—	—	Artificial perfusion started, <i>in situ</i> Isolation of liver complete
2.55	.77	.48	.21	—	—	
3.25	.70	.39	.21	—	—	Two fresh lobes 0.75 mgr. adrenaline introduced into lungs-reservoir
3.55	.75	.45	.35	.26	.20	
4.25	.68	—	—	.38	.36	
4.55	.83	—	—	.08	.13	

Exp. 3. Liver perfused with 1550 c.c. blood containing 30 grm. glucose.

Time	Per-centage blood sugar	Percentage liver glycogen				
		I lobe	II lobe	III lobe	IV lobe	
2.50	2.09	.36	.43	—	—	Arterial perfusion started, <i>in situ</i> Portal perfusion started Isolation of liver complete
2.55	—	—	—	—	—	
3.30	1.84	.24	.41	—	—	Two fresh lobes 0.75 mgr. adrenaline introduced into portal reservoir
4.0	1.66	.21	.42	—	—	
4.30	1.66	.35	.45	.28	.29	
5.0	1.50	—	—	.38	.52	
5.30	1.50	—	—	.55	.59	
6.0	1.46	—	—	.21	.27	

and the blood sugar determined by the method of Hagedorn-Jensen. The insulin used was a sample of dry hydrochloride containing 8 units per mgr., which was dissolved in a small quantity of blood just before use. The liver was weighed at the end of the experiment.

In all these experiments, which were performed towards the end of the investigation, all the precautions previously referred to were strictly observed. Since the first samples were taken from the liver still *in situ*, at the time of starting the arterial perfusion, it was found impossible to take all the subsequent samples from the same two lobes, as we did not wish to take our samples from the part of the lobe already cut. One hour after the isolation of the liver, therefore, two fresh lobes were started, so that at this point four samples were actually taken. In Exps. 1 and 2 it will be observed that there is a considerable breakdown of glycogen between the taking of samples I and II, *i.e.*, during the period of preparation and isolation of the liver. We hoped to prevent this by starting also the portal circulation with the liver *in situ*, but as will be seen from Exp. 3, not with complete success. For, although the glycogen content of the second lobe remained practically constant, that of the first lobe showed a decrease. It is unfortunate that in this experiment the initial glycogen content was low.

Considering now the perfusion proper, after complete isolation of the liver, a deposition of glycogen is regularly observed, except in the case of the second lobe in Exp. 1, and the first lobe in Exp. 2, where the initial breakdown would appear to extend into the first half-hour of perfusion, so that glycogen storage is not observed until later.

It is striking that the fresh lobes, from which no samples were taken until one hour after the isolation, contained considerably less glycogen than the two first lobes at the same time. The reason for this is not clear. Possibly the deposition of glycogen is facilitated by the increased flow of blood through the lobes which have already been cut.

In Exps. 2 and 3 adrenaline was added to the perfusing blood during the last half-hour. In Exp. 2, where the adrenaline entered the liver both by the hepatic artery and the portal vein, a considerable rise in arterial pressure took place, whereas when the adrenaline was introduced only by the portal route, as in Exp. 3, the rise was very much smaller. In both cases, however, a well-marked breakdown of glycogen was observed.

In practically all our experiments the blood sugar showed little change.

*Experiments with insulin.*

*Exp. 4.* Liver weighing 355 gm. perfused with 1750 c.c. of blood.

		Hepatic artery		Portal vein
Pressure ...		100 mm.		15 mm.
Temperature ...		38-39° C.		38-39° C.
		Percentage liver glycogen		
		I	II	
Time	Per-centage blood sugar	lobe	lobe	
4.20	—	.47	.65	Isolation of liver complete 25 gm. glucose added
4.25	—	—	—	
4.30	1.33	—	—	
5.0	1.22	.58	.84	
5.40	1.22	.57	.91	Ten units of insulin introduced into lungs-reservoir Eight units of insulin
6.20	1.21	.27	.67	
7.0	1.20	.35	.42	

*Exp. 5.* Liver weighing 413 gm. perfused with 1600 c.c. blood containing 30 gm. glucose.

		Percentage liver glycogen				
		I	II	III	IV	
Time	Per-centage blood sugar	lobe	lobe	lobe	lobe	
2.35	—	.32	.34	—	—	<i>In situ</i> , arterial perfusion started Portal perfusion started Isolation of liver complete
2.40	—	—	—	—	—	
3.5	1.34	.10	.16	—	—	
3.35	1.20	.20	.21	—	—	
4.5	1.34	.32	.29	.23	.15	Five units of insulin added to portal reservoir
4.35	1.22	—	—	.25	.15	
5.5	—	—	—	.18	.15	Five units of insulin added to portal reservoir 0.75 mgr. adrenaline added to portal reservoir
5.35	1.45	—	—	.09	.06	

*Exp. 6.* Liver weighing 290 gm. perfused with 1500 c.c. blood containing 30 gm. glucose.  
Arterial temperature fell to 35° C. during first half-hour, due to slow arterial flow.  
Later raised to 38° C.

		Percentage liver glycogen		
		I	II	
Time	Per-centage blood sugar	lobe	lobe	
4.30	1.95	3.72	3.30	Isolation of liver complete
5.0	1.97	2.60	2.50	
5.30	1.90	2.78	2.99	Ten units of insulin added to lungs-reservoir Five units of insulin added to lungs-reservoir
6.0	1.96	3.04	2.71	
6.30	2.11	3.01	3.38	

*Exp. 7.* Liver weighing 365 gm. perfused with 1500 c.c. blood containing 30 gm. glucose.

		Percentage liver glycogen		
		I	II	
Time	Per-centage blood sugar	lobe	lobe	
3.55	1.76	.027	.019	Isolation of liver complete
4.25	1.69	.022	.028	
4.55	1.72	.029	—	Ten units of insulin added to lungs-reservoir Five units of insulin added to lungs-reservoir
5.25	1.76	.022	.026	
5.55	1.69	.024	.024	

In Exp. 4, where the first samples were taken only after isolation of the liver, and the same lobes were used throughout, a well-marked storage is observed during the first hour control period, which gives place to a breakdown after the addition of insulin.

In Exp. 5, in which the first samples were taken from the liver still *in situ*, and the portal circulation was interrupted for only a short time, a loss of glycogen is still observed during the period of preparation and isolation of the liver. During the first hour control period there is a storage of glycogen, whereas in the second hour insulin, in contrast to Exp. 4, merely stops the further deposition of glycogen. It is also seen that the samples from fresh lobes, taken at the beginning of the insulin period, contained less glycogen than the later samples from the first lobes taken at the same time.

In Exp. 6, apart from the breakdown of glycogen observed during the first half-hour, due no doubt to the accidental fall in temperature of the blood, a steady increase in glycogen was observed, which was unaffected by the introduction of insulin. This is the only experiment in which we have observed an increase in glycogen to occur during the insulin period. We have never been able to repeat it, and have no explanation to offer for it. It is worthy of note that the liver contained a high percentage of glycogen, as has also been observed in a few other experiments. We do not know the reason for this, but it presumably depends upon the state of nutrition of the animal.

Exp. 7 is given as an example of the effect of deviating from the procedure previously laid down. In this case too much ether was administered and the heart stopped before the arterial perfusion *in situ* could be started. The initial glycogen content was low, and remained practically unchanged throughout the experiment.

In contrast to the results of Bornstein and Griesbach on the one hand, and of von Issekutz on the other, in Exp. 5, the adrenaline given one hour after insulin is observed to cause just as big a breakdown of glycogen as in the absence of insulin.

#### EXPERIMENTS WITH FASTING AND FAT-FED DOGS.

In the foregoing experiments insulin is observed either to stop the storage of glycogen, or to cause a breakdown, with the exception of Exp. 6, in which it apparently had no effect. This seems to be the immediate effect of insulin, whether given by the portal vein or by the hepatic artery as well. Further, in all the control periods of our experiments a glycogen storage was observed, but we were unable to exclude

the possibility that this storage might be due to the presence of insulin in the liver cells during life, and still active in the isolated liver. In an attempt to obtain a liver free from insulin, we first carried out experiments on dogs fasting for 48 hours, and then on animals maintained for five days on a fat diet.

*Exp. 8.* Dogs fasting for 48 hr. Liver weighing 380 gm. perfused with 1400 c.c. blood containing 25 gm. glucose.

		Hepatic artery		Portal vein
Pressure ...		...	110 mm.	15 mm.
Temperature ...		...	39° C.	38.5° C.
		Percentage liver glycogen		
		I	II	
Time	Per-centage blood sugar	lobe	lobe	
3 50	—	.023	.015	Isolation of liver complete
4.20	1.43	.045	.057	
4.50	1.39	.095	.074	Seven units of insulin added to lungs reservoir.
5 20	1.43	.060	.064	Five units of insulin added to lungs-reservoir
5 50	1.27	.040	.026	

*Exp. 9.* Dogs fasting for 48 hr. Liver weighing 500 gm. perfused with 1450 c.c. of blood containing 30 gm. glucose.

		Hepatic artery		Portal vein
Pressure ..		...	100 mm.	15 mm.
Temperature ...		..	39.0° C.	38.3° C.
		Percentage liver glycogen		
		I	II	
Time	Per-centage blood sugar	lobe	lobe	
3 0	1.79	.031	.020	Isolation of liver complete
3 30	1.73	.042	.046	
4.0	1.73	.154	.083	Six units of insulin added to lungs reservoir
4.30	1.73	.070	.055	Five units of insulin added to lungs reservoir
4.50	1.64	.063	—	

*Exp. 10.* Dogs fed on horse fat for five days Liver weighing 550 gm. perfused with 1400 c.c. of blood. No glucose.

		Hepatic artery		Portal vein
Pressure ..		..	105 mm.	15 mm.
Temperature ..		.	38.8° C.	38.3° C.
		Percentage liver glycogen		
		I	II	
Time	Per-centage blood sugar	lobe	lobe	
2 10	.139	—	—	Arterial perfusion started
2 35	.195	.018	.018	Isolation of liver complete
3 5	.236	.015	.020	
3 35	.247	.023	.024	Six units of insulin added to lungs reservoir
4.5	.271	.021	.020	Six units of insulin added to lungs reservoir
4.35	.298	.022	.022	

Exp. 11. Dogs fed on horse fat for five days. Liver weighing 245 grm. perfused with 1350 c.c. of blood containing 25 grm. glucose.

		Hepatic artery		Portal vein		
Pressure ...		... 100-60 mm.		15 mm.		
Temperature ...		... 38.1° C.		38.9° C.		
Time	Per-centage blood sugar	Percentage liver glycogen				
		I lobe	II lobe	III lobe	IV lobe	
12.35	—	1.44	.92	—	—	Arterial perfusion started, <i>in situ</i> Isolation of liver complete
1.20	1.90	.30	.20	—	—	
1.50	1.79	.31	.23	—	—	Five units of insulin added to lungs-reservoir
2.20	1.82	.33	.32	.41	.28	
2.50	1.81	—	—	.35	.16	Five units of insulin added to lungs-reservoir
3.20	—	—	—	.21	.16	

Arterial perfusion started, *in situ*  
Isolation of liver complete

Five units of insulin added to lungs-reservoir  
Five units of insulin added to lungs-reservoir

Exps. 8 and 9 were performed on fasting animals, and differed only in the addition of glucose to the blood in Exp. 9. In Exp. 8 the initial glycogen content of the liver was low and showed only a slight increase during the course of the experiment. In Exp. 9 the increase in glycogen content was somewhat greater, but in both experiments the effect of insulin was to cause a breakdown of glycogen.

Exps. 10 and 11 were performed on fat-fed animals, again with the difference that the sugar was added to the blood in Exp. 11. Again in Exp. 10, the initial glycogen content is very low, and remains unchanged during the experiment. In Exp. 11, on the other hand, the general level of glycogen is much higher and, apart from the breakdown occurring during the preparation and isolation of the liver, there is during the control period an increase in the glycogen content of one lobe, while that of the other remains constant. Insulin causes a breakdown of glycogen. It is interesting to note that the liver of a dog fed exclusively on fat for five days may contain as much glycogen as in some of our experiments on normal animals.

It will be observed that in Exp. 10, in corroboration of the previous results of Burn and Marks(a), the blood sugar shows a progressive increase during the course of the experiment. Further, this increase is unaffected by the addition of insulin. According to Burn and Marks, this increase demonstrates the new formation of sugar by the liver of the fat-fed animal. It remains to be proved that the increase in reducing power observed really represents an increase in glucose content. If it does, we see that insulin has no immediate effect on new formation of sugar in the liver under the conditions of these experiments. In the paper cited above evidence is given of a glycogen storage in the liver of

fat-fed cats which we failed to obtain in the above experiment. This is probably to be attributed to the fact, observed also by other workers, that glycogen storage is more readily obtained in cats than in dogs. Thus von Barrenscheen<sup>(12)</sup> was able to obtain glycogen storage in the livers of dogs only when the perfusing blood contained at least 1 p.c. of sugar.

#### DISCUSSION.

Von Barrenscheen<sup>(12)</sup>, working in Hofmeister's laboratory, appears to have been the only observer who succeeded in demonstrating a glycogen storage in the perfused liver of the dog, and that only during the first forty-five minutes of his perfusion. He already drew attention to the importance of certain factors, namely, the use of as little ether as possible, the careful regulation of temperature, the presence of an excess of sugar in the blood, and the necessity of isolating the liver as quickly as possible. He obtained good results only when he was able to prepare and isolate the liver within five minutes of bleeding out the animal. This means that asphyxiation of the liver was reduced to a minimum.

Our experiments bear out the opinions of von Barrenscheen, but we feel that we have in some ways improved upon his technique. Thus, by perfusing through both the hepatic artery and portal vein under appropriate physiological pressures, instead of perfusing through the portal vein only, under the abnormally high pressure employed by previous workers, and, further, by the use of the lungs for the removal of the vaso-constrictor property of the defibrinated blood, we were able to perfuse the liver for two or three hours without the slightest sign of liver oedema at the end of our experiment. Also, since we were able to establish the artificial double perfusion of the liver while it still received part of its natural blood supply, the organ was not asphyxiated for even a short time. Under these more nearly physiological conditions we were able to obtain a more pronounced glycogen storage.

It is true that in most of our experiments the glycogen content of the liver was not very high, but we attribute this to the use of dogs, which was necessary in order to have available the requisite amount of blood. Probably the cat's liver would have been more suitable for such experiments, but it would have necessitated the use of a large number of animals for each experiment, or else perfusion with the blood of another species, which we did not consider to be physiological.

Insulin, whether given by the portal vein, or by the hepatic artery as well, either caused a breakdown of glycogen or prevented further storage, except in one experiment.

Adrenaline caused a rapid breakdown of glycogen, but, contrary to the experience of Bornstein and Griesbach, and of von Issekutz, this was quite unaffected by insulin, given previously. Von Issekutz suggested that glycogenolysis was largely determined by acid production in the perfused liver. In his experiments, in which the liver was perfused with Ringer's solution, the question of acid production was no doubt of considerable importance. In our experiments, using a large volume of blood, which is a much more efficient buffer than Ringer's solution, and effects a much more efficient oxygenation, we feel that the production of acid is of less consequence. We have not investigated the acid production, but whether it occurs or not, our control experiments demonstrate that glycogen is stored.

The evidence we have obtained indicates that the immediate and direct effect of insulin, at any rate, is not to promote glycogen storage in the liver, under the conditions of our experiments. It is still possible, of course, that increased deposition of glycogen in the liver may occur several hours after the administration of insulin to the whole animal, as, indeed, is affirmed by von Issekutz. We hope later to investigate this possibility.

On the other hand, we observed a definite storage of glycogen in the absence of added insulin, which might possibly be due to the presence in the cells of the isolated liver of insulin previously secreted by the pancreas *in vivo*. In an attempt to exclude this possibility we performed experiments, first on fasted and then on fat-fed animals. In these experiments, when extra glucose was added, we still observed a glycogen storage without the addition of insulin, while when insulin was added a breakdown of glycogen took place, just as in the case of normal animals. It may be that the only way to ensure the complete absence of insulin is by performing experiments on depancreatized animals, and this we hope to do in the near future.

In one of our experiments on fat-fed animals, in which the steady rise of blood sugar without loss of glycogen from the liver indicated a rapid new formation of sugar in that organ, the process was apparently unaffected by adding insulin to the perfusing blood. On the basis of a comparison of the observed action of insulin on the whole animal with that on an animal in which the liver has been excluded from the circulation, it has been suggested, in a previous communication from this Institute<sup>(13)</sup>, that an important part of the action of insulin consists in a stoppage of new formation of carbohydrate in the liver. The experiments on the livers of fat-fed animals recorded above, however, afford no



evidence in support of this view, and we are led to suspect that the liver, when isolated and perfused, does not respond to insulin in the same way as when it is performing its physiological functions in the whole animal. It may be that the co-operation of other organs is necessary in order that the liver may exhibit its true physiological response to insulin. This also may be the reason why we have failed to observe an increased deposition of glycogen as a result of administering insulin to the isolated perfused liver.

#### SUMMARY.

1. A new method for the perfusion of the isolated mammalian liver is described, perfusing through both the hepatic artery and the portal vein under physiological pressures, without interruption of the blood supply.

2. Glycogen storage in the absence of added insulin was observed.

3. Added insulin either stopped further storage or caused a breakdown of glycogen.

4. Addition of adrenaline caused a rapid breakdown of glycogen.

5. This was not abolished by the previous addition of insulin.

6. The livers of starved and of fat-fed animals showed the same behaviour.

7. The new formation of sugar by the liver of the fat-fed animal was not affected by addition of insulin.

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# THE FACTORS INFLUENCING THE CONCENTRATION OF HYDROCHLORIC ACID DURING GASTRIC DIGESTION.

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IN recent years a very important part has been attributed to the regurgitation of alkaline intestinal contents into the stomach during digestion. That some regurgitation does occasionally take place is certain, but evidence is brought forward in this paper which shows that any regurgitation which may occur plays but a very insignificant part in the physiology of digestion.

The presence of bile in the contents of the stomach has often been observed. It has perhaps been most frequently noted in vomited matter in which it sometimes occurs in large amount, but it is by no means rare to find it in specimens obtained from the stomach by means of a tube. Little, however, was known with regard to the significance of bile in the stomach until Boldyrev(1) undertook an investigation of this question. He confirmed an earlier observation by Pavlov that the introduction of oil into the stomach gave rise to a regurgitation of bile from the intestine; he also found that the introduction of mineral acids into the resting stomach provoked a regurgitation of alkaline duodenal fluid which reduced the acidity to a level (0.15 to 0.20 p.c.) at which it was acceptable to the intestine. Boldyrev's work was in general supported by Cathcart(2) and later by Carlson(3) and other observers.

Before the publication of Boldyrev's experiments the regurgitation of intestinal contents into the stomach was regarded more or less as a pathological phenomenon, but the work of this observer seemed to give it a definite and important place in the chain of events occurring during gastric digestion, and led to the view that the concentration of hydrochloric acid in the stomach was controlled by this mechanism.

After the introduction of the fractional method of gastric analysis by Rehfuess many observations were made bearing on this conception, and, on the whole, Boldyrev's conclusions were sustained. Bolton and Goodhart(4), employing this method, estimated at intervals of fifteen

minutes hydrochloric acid as well as total chloride and neutral chloride. They found, in common with other observers, that the hydrochloric acid rose to a certain height during digestion and then fell, the steepness of both rise and fall and the height of the curve being subject to considerable variations even in normal individuals. Fig. 1 shows a typical result ob-

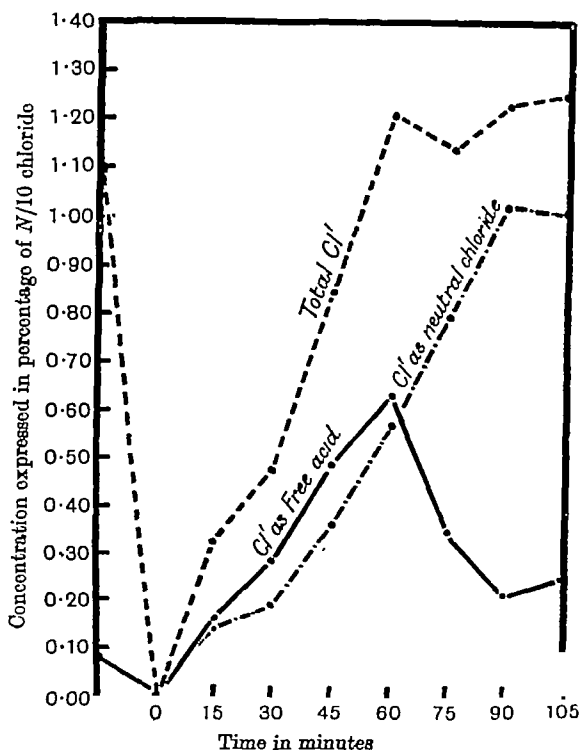


Fig. 1. Curves showing variations in hydrochloric acid, neutral chloride and total chloride after a test meal in a normal subject.

tained by this method in a healthy person. The output of neutral chloride increased, somewhat less than the acidity, during the period in which the acidity was increasing, but when the acidity fell and as long as it continued to fall there was a marked rise in neutral chloride.

Observations such as these were explained thus: at a certain point during digestion, when the curve of acidity was rising, the pylorus relaxed and allowed duodenal fluid to enter the stomach, thus reducing the acidity and correspondingly increasing the amount of sodium chloride.

In various pathological conditions of the stomach these workers found

that there was a general relationship between the amount of neutral chloride and the concentration of acid; when the amount of acid remained high the neutral chloride remained low; when the neutral chloride was high the acid was comparatively low. They came to the conclusion that the general form of the curves obtained depended upon the amount of regurgitation, and was an index of the behaviour of the pyloric sphincter; at the present time this is the generally accepted theory.

As the result of many investigations on the human subject it became apparent to us that this theory of regurgitation did not account for the facts, and that it was possible to place another interpretation on the curves described.

In our experiments fractional test meals were used, consisting of a small amount of shredded wheat in about twenty ounces of distilled water. Before giving the meal the subject was asked to swallow a small stomach tube. By careful aspiration as much of the resting juice as possible was removed from the stomach. This is an important precaution. The meal was then given without removing the tube from the stomach. The subject was told not to swallow any saliva and was provided with a receptacle in which to collect it. As during the period of investigation some individuals secrete 150 c.c. or more of saliva, this is also important.

Fractions consisting of about 10 c.c. were withdrawn at intervals by a syringe, delivered into special tubes, centrifuged and investigated as described below. In our experience, centrifugal separation is much preferable to filtration.

In very many normal subjects, although no bile was detectable in the stomach, when the acidity of the contents fell the neutral chloride rose. Provided that we can suppose it possible for pancreatic fluid to enter the stomach unaccompanied by bile, this chloride might be supplied by the secretion of the pancreas, which as Carl Schmidt showed contains chloride up to 0.7 p.c.

If pancreatic fluid does enter the stomach in any appreciable amount, the interaction of pancreatic alkali (sodium bicarbonate) with hydrochloric acid would lead to the liberation of carbon dioxide, and it should be possible to show its presence. In addition, proof of the presence of trypsin in the stomach would be valuable evidence in favour of regurgitation of pancreatic fluid.

In what follows we describe experiments in which there was no obvious regurgitation, as indicated by the presence of bile in the fluid removed, but in which the gastric acidity followed the same course as when bile

is found. In our experience the acidity curve has the same form in either case, whether bile is present or not.

*Estimation of  $\text{CO}_2$  in fractional samples of gastric contents.* If fluid containing bicarbonates should enter the stomach when this contains hydrochloric acid clearly carbonic acid would be liberated; collecting in the gas over the fluid contents this would raise the partial pressure of  $\text{CO}_2$  in it, and consequently a larger amount would also be dissolved in the fluid, in simple proportion to this partial pressure so long as the fluid remained acid. But since equilibrium between the  $\text{CO}_2$  in the fluid and in the gas over it would probably not be established instantly, if samples of the fluid could be removed soon enough after the reaction between bicarbonate and acid, quantities of  $\text{CO}_2$  might be found in the fluid even larger than corresponded to this equilibrium. If the amount of bicarbonate was more than sufficient to react with all the hydrochloric acid then of course still larger amounts of  $\text{CO}_2$  would be found in the fluid. Estimation of carbon dioxide under the conditions of the experiment proved to be a somewhat difficult problem. When sucked up by a syringe in the ordinary way the gastric fluid is subjected to a considerably reduced pressure, which favours the escape of  $\text{CO}_2$  from the fluid. A method had to be devised whereby it was possible to ensure that any gas which escaped in this manner was collected and estimated. In addition, the gastric fluid had to be preserved from exposure to the atmosphere.

*Description of apparatus.* The apparatus which was accordingly employed is shown in Fig. 2. *A* is the syringe and *B* the stomach tube. *C* is a centrifuge tube which fits on to a band of rubber on *D*. *E* is a small reservoir of paraffin. *F* contains about 10 c.c. of 40 p.c. caustic soda. *G* is an ordinary Bunsen valve placed so as to prevent any liquid from passing back from tube *H*, which contains 5 c.c. of  $N/2$  sodium hydroxide covered by a layer of paraffin.

Before carrying out an experiment a number of clean dry tubes were taken and into each was delivered 5 c.c. of  $N/2$  sodium hydroxide, the surface of the liquid being immediately covered by a thin layer of paraffin. When preparing to remove a sample from the stomach a centrifuge tube was attached to *D*, *I* was closed, and *J* turned to communicate with *F*. By withdrawing the plunger of the syringe pure air was sucked into the apparatus and afterwards expelled by turning *J* to communicate with *D*. This operation was repeated once or twice to displace the air in the apparatus with  $\text{CO}_2$ -free air.

Tube *H*, containing the measured amount of sodium hydroxide, was then attached. The stomach tube was then connected with the apparatus and, with *J* closed and *I* opened, sufficient gastric fluid was drawn into the syringe. Tap *I* was now closed again and *J* turned to communicate with *C*, when the fluid was gently expelled from the syringe into *C*. *J* was reversed, the syringe filled with air from *F*, *J* again reversed and the air driven through the apparatus, removing at the same time any fluid remaining in the tubing between *A* and *C*. A little paraffin was allowed to run from *E* on to the surface of the fluid in *C* so as to cover it completely.

The apparatus was then swept through with pure air once or twice in the manner

described above. Since all the air passes through the sodium hydroxide in *H* any carbon dioxide which escaped from the fluid then in *C* was trapped. For the next fraction the tubes *C* and *H* were changed and the operations repeated.

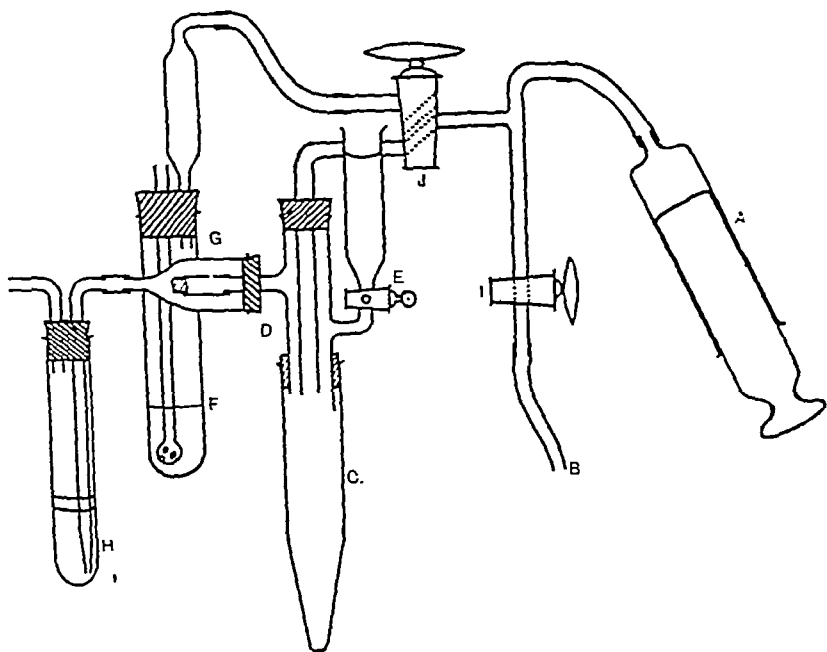


Fig. 2. Apparatus for obtaining samples of gastric fluid without loss of  $\text{CO}_2$ .

As it was necessary to know the volume of fluid removed, graduated centrifuge tubes were used, or the level was marked on the tube and the volume determined subsequently.

The amount of carbon dioxide in the gastric fluid and in the  $\text{N}/2$  sodium hydroxide in *H* was determined by the method of Van Slyke<sup>15</sup>. Generally 2 c.c. of the fluid (gastric fluid or  $\text{NaOH}$ ) were introduced into the apparatus and washed in with two portions of 0.5 c.c. of water. To ensure complete acidity, 2 c.c. of 2 *N* hydrochloric acid were added before extraction. After extraction, the volume of gas was recorded; the carbon dioxide was absorbed by 0.5 c.c. of  $\text{N}/2$  sodium hydroxide and the volume of air remaining read off; the difference between these readings is the amount of carbon dioxide.

The precautions necessary when carrying out such work were strictly observed. Pipettes were not blown into, and when dealing with sodium hydroxide we found it advisable to coat the outer surface of the ends of pipettes with a thin film of vaseline to reduce the amount of alkali left adhering and exposed to the atmosphere.

Since the  $\text{N}/2$  sodium hydroxide used invariably contained traces of carbonate, a blank determination was carried out and the necessary correction applied to the results.

For each sample of gastric contents removed two results were obtained: (1) the total carbon dioxide in the 5 c.c. of sodium hydroxide, (2) the concentration of carbon dioxide in the fluid itself. The carbon dioxide represented by (1) is derived from the volume of gastric fluid removed; this volume being known, the percentage of carbon dioxide was calculated and added to (2).

*The CO<sub>2</sub> in the fluid from the stomach.* Preliminary experiments were carried out in order to determine the effect of introducing sodium bicarbonate into the stomach during gastric activity. Forty minutes after taking a test meal and with the tube in the stomach 0.2 gram. of sodium bicarbonate in 5 c.c. of water was taken and washed down with a small quantity of water. In a sample obtained five minutes afterwards there was an increase in the amount of carbon dioxide from 2 c.c. to 32 c.c. per 100 c.c. gastric contents, and the amount of neutral chloride increased.

On another occasion 0.05 gram. of sodium bicarbonate in 4 c.c. of water was swallowed at intervals, sometimes before, sometimes after removing a sample from the stomach. It will be seen (Table I) that even this small amount of alkali (equivalent to 6 c.c. of N/10 sodium bicarbonate) is capable of producing a marked increase in dissolved carbon dioxide.

TABLE I. Showing effect of ingested bicarbonate on CO<sub>2</sub> of gastric contents.

Time in minutes after taking meal	Bicarbonate given	Total Cl' N/10	Neutral Cl' N/10	HCl N/10	CO <sub>2</sub> in c.c. per 100 c.c. fluid
13	0.05 gram. NaHCO <sub>3</sub>	—	—	—	—
15	—	0.319	0.312	0.007	29
30	—	0.344	0.142	0.202	10
32	0.05 gram. NaHCO <sub>3</sub>	—	—	—	—
45	—	0.468	0.262	0.206	11
58	0.05 gram. NaHCO <sub>3</sub>	—	—	—	—
60	—	0.660	0.414	0.246	26
75	—	0.820	0.341	0.479	6
77	0.05 gram. NaHCO <sub>3</sub>	—	—	—	—
95	—	1.040	0.433	0.607	15
105	—	0.991	0.312	0.679	—

Having thus determined the possibility of detecting the entrance of small quantities of bicarbonate into the stomach, we proceeded to examine the gastric fluid after an ordinary test meal in many experiments on healthy subjects. We found that there was constantly a small amount of carbon dioxide dissolved in the gastric fluid averaging from 3 to 10 c.c. per 100 c.c. of fluid. Such variations as occurred were small, and bore no relation whatever to the curves of acidity and neutral chlorides. In a few cases, at the end of the experiment when a fair amount of bile was present, the CO<sub>2</sub> of the contents increased, but we were always unable to obtain any evidence in favour of regurgitation of pancreatic fluid in the absence of bile either as an intermittent phenomenon or as one occurring at a definite point in gastric activity. Sometimes, indeed, the CO<sub>2</sub> content

was higher when the hydrochloric acid was increasing rapidly, and considerably lower when neutral chloride was rising and acidity decreasing.

Table II shows the results obtained in three normal subjects.

TABLE II. Showing amount of CO<sub>2</sub> obtained from fractional samples of gastric contents obtained from normal subjects.

Exp.	Time in minutes	Bile	Total Cl' N/10	Neutral Cl' N/10	HCl N/10	CO <sub>2</sub> in c.c. per 100 c.c. fluid
1	15	Absent	0.302	0.227	0.075	3.1
	30	"	0.647	0.332	0.315	3.4
	45	"	0.922	0.479	0.443	5.8
	60	"	1.270	0.655	0.615	6.8
	75	"	1.230	0.928	0.305	6.5
	105	"	1.270	0.998	0.274	7.6
	120	"	1.260	1.130	0.128	5.5
2	15	Absent	0.203	0.132	0.071	2.8
	30	"	—	—	—	2.9
	45	"	0.776	0.485	0.291	2.1
	60	"	0.947	0.540	0.407	3.0
	75	"	1.120	0.638	0.482	2.6
	90	"	1.240	0.851	0.384	4.2
	105	"	1.240	1.050	0.160	3.8
3	120	Present	1.090	1.060	0.030	11.4
	30	Absent	0.350	0.148	0.202	11.0
	45	"	0.573	0.324	0.249	8.2
	60	"	0.733	0.334	0.401	7.5
	75	"	0.814	0.468	0.346	8.2
	90	Present	0.916	0.680	0.236	13.0

In Exps. 1 and 2 it will be seen that the amount of CO<sub>2</sub> in the gastric contents did not vary to any appreciable extent during the whole period of digestion. In both these subjects the hydrochloric acid reached a maximum in approximately one hour, and then gradually declined while the neutral chloride steadily rose. This marked decrease in hydrochloric acid caused no increase in dissolved carbon dioxide. In some other experiments the average amount of CO<sub>2</sub> found was considerably higher and more erratic, but it never became consistently higher during the decrease of HCl and the increase of neutral chloride. On the other hand the presence of bile in Exp. 2 coincided with a definite increase in CO<sub>2</sub> content, and whenever bile was present in considerable amount the CO<sub>2</sub> content of the gastric fluid increased.

*The tryptic activity of the gastric contents.* Regurgitation of pancreatic secretion during digestion should result in the presence of trypsin in the stomach, which should increase during the later stages of gastric activity when the hydrochloric acid is decreasing, if this decrease is due to neutralisation by fluid from the intestine. We carried out a number of experiments with a view to determining whether any significant amount of trypsin is actually present in the gastric contents during digestion and



more particularly during the period of falling acidity. Spencer, Meyer, Rehfuß and Hawk<sup>(6)</sup> examined specimens of gastric contents after different meals for trypsin at different stages of digestion. They found that a tryptic enzyme was almost constantly present in the fasting and digesting stomach, and that the activity of this enzyme varied inversely as the acid content, usually following the colour changes due to bile. Hydrochloric acid and sodium bicarbonate when introduced into the stomach by mouth provoked regurgitation of bile accompanied by trypsin. The relationship between tryptic activity and acidity in these experiments cannot be said to be very distinct, nor do they throw much light on the question of regurgitation in the absence of bile.

*Estimation of trypsin.* After various attempts to estimate small amounts of trypsin the following method was adopted. A quantity (1 to 2 c.c.) of the centrifuged gastric contents was measured into a test-tube graduated at 10 c.c. After adding one drop of methyl red, the fluid was made faintly alkaline with *N*/10 sodium hydroxide. The reaction was then adjusted to a faint pink by cautious addition of *N*/100 sulphuric acid. Five c.c. of casein solution were added and the volume made up to 10 c.c. with water. The contents of the tube were thoroughly mixed by shaking.

Two dry test-tubes were taken and into each 4 c.c. of this mixture were delivered. One portion which served as a control was brought to the boiling point and cooled. Both tubes were heated for two hours at 40° C. in a water bath. When incubation was complete the tubes were removed from the bath and to each was added 1.5 c.c. of 25 p.c. trichlor-acetic acid whereby the undigested protein was precipitated. The mixture was filtered through a Whatman No. 1 filter paper, the first few drops being refiltered if necessary. It is essential that the filtrate should be perfectly clear. The total nitrogen in 4 c.c. of this filtrate was determined by the method of Folin and Denis, *N*/100 acid and alkali being employed.

The casein solution was prepared as follows: two grams of pure casein were dissolved in a boiling solution of 17 c.c. of *N*/10 sodium hydroxide in 180 c.c. of water. Solution was facilitated by first rubbing the casein into a thin cream with a little of the alkali. The solution was filtered hot and the opalescent filtrate made up to 200 c.c. with water.

Bile prevents complete precipitation of the protein by trichlor-acetic acid, a colloidal solution being obtained which will not yield a clear filtrate. When bile is present the sample must be diluted five to ten times before proceeding with the estimation.

The tryptic activity was given by the amount of nitrogen in the soluble products yielded by 1 c.c. of alkaline gastric fluid after incubation, measured as c.c. of *N*/100 acid, allowance being made for the control. When the original fluid was diluted before estimation the tryptic activity was assumed to be proportional to the dilution. The method proved to be a very delicate one and gave definite results with very small amounts of trypsin.

*Results.* Table III shows the results obtained in various subjects. In the resting juice considerable amounts of trypsin were present as might be expected. During gastric activity in some subjects a definite amount of trypsin was found, but only when bile was present. In all others only indefinite traces of trypsin were found notwithstanding a marked rise in neutral chlorides from the beginning and a definite fall in acidity subsequently. No relationship between the variations in

TABLE III. Showing tryptic activity of fractional samples of gastric contents.

Exp 1 was done on a subject suffering from alcoholic gastritis, the others on normal subjects.

Exp.	Time	Bile	Total Cl' N/10	Neutral Cl' N/10	HCl N/10	Trypsin units
1	Resting	—	0.018	0.618	0.000	8.5
	15 min.	—	0.154	0.128	0.026	3.6
	30 "	—	0.166	0.134	0.032	0.5
	45 "	—	0.244	0.204	0.040	1.4
	60 "	—	0.302	0.250	0.052	0.9
	75 "	—	0.442	0.410	0.032	4.3
	90 "	—	0.608	0.643	0.025	2.7
2	Resting	—	—	—	—	—
	30 min.	—	0.371	0.255	0.116	1.4
	45 "	—	0.642	0.438	0.204	0.0
	60 "	—	1.040	0.634	0.405	0.0
	75 "	—	1.150	0.851	0.298	0.9
	90 "	—	0.965	0.890	0.075	0.0
3	Resting	+	1.060	—	—	79.0
	30 min.	—	0.344	0.239	0.105	0.0
	45 "	—	0.698	0.488	0.210	2.8
	60 "	—	0.892	0.557	0.335	2.8
	75 "	—	1.080	0.690	0.390	1.6
	90 "	—	1.210	0.961	0.250	0.0
	105 "	—	1.220	1.100	0.120	0.0
4	Resting	trace	1.070	0.560	0.510	—
	15 min.	—	0.177	0.117	0.060	—
	30 "	—	0.376	0.121	0.255	5.3
	45 "	—	0.421	0.131	0.290	—
	60 "	—	0.579	0.099	0.480	0.7
	75 "	—	0.877	0.467	0.410	—
	90 "	—	0.996	0.614	0.382	0.0
	105 "	—	0.907	0.642	0.265	2.0
5	Resting	+	0.973	—	—	99.0
	15 min.	—	0.053	0.034	0.019	0.5
	30 "	—	0.108	0.060	0.048	—
	45 "	—	0.334	0.262	0.072	0.5
	60 "	—	0.489	0.342	0.147	—
	75 "	—	0.594	0.440	0.154	0.7
	90 "	—	0.726	0.564	0.162	—
	105 "	—	0.921	0.638	0.283	0.0
	120 "	—	0.996	0.704	0.292	0.0

tryptic activity and the curves of acidity was found in any subject. These experiments show very clearly that, in the absence of bile, it is rare to find more than minimal amounts of trypsin in the stomach during digestion; often it is quite absent. On one occasion, however, we did obtain a sample of resting juice which had a fair tryptic activity although containing no bile. Associated with bile the tryptic activity was always found to be considerable.

*Experiments with a test meal containing acid.* Further evidence on the question whether alkaline fluid from the intestine enters the stomach during digestion was sought in the following way. If in subjects who secrete no hydrochloric acid an acid solution is introduced into

the stomach, acid sodium sulphate for instance, together with a test meal, the entry of an alkaline fluid through the pylorus would change the ratio of acid sulphate to total sulphate, whereas dilution with gastric juice, free from acid, would lower the concentration both of acid and total sulphate without altering this ratio.

The first experiment on these lines was carried out on a young female subject in whom a fractional test meal showed entire absence of hydrochloric acid, both free and combined, with a definite secretion of neutral chloride. A meal was given consisting of 370 c.c. of wheat-meal fluid together with 188 c.c. of sodium hydrogen sulphate solution prepared by adding 50 c.c. of normal NaOH to 100 c.c. of normal  $\text{H}_2\text{SO}_4$  and 50 c.c. of distilled water. This mixture is too acid and unpleasant to drink, so it was injected into the stomach through a tube.

The acidity of samples from the stomach was determined by titration; total chloride, sulphate and trypsin were also determined in each fraction.

During the first hour and a quarter the acidity was reduced to roughly one-half of its initial value. As however the concentration of sulphate also fell and the ratio of titratable acidity to total sulphate was not changed, the acidity must have been reduced not by neutralisation but by dilution. After little more than two hours the acidity was still further reduced with only a very slight change in the ratio of acid to total sulphate, not more than could be accounted for by the secretion of alkaline mucus in the samples. The resting juice of this patient contained much bile and trypsin, but fractions removed after a meal was taken showed a small and decreasing amount of trypsin probably derived from a small residue of resting juice left in the stomach. There was no evidence of an increase of trypsin such as would be likely to accompany regurgitation. The curve of neutral chloride closely resembled a normal curve.

The next experiment of this nature was carried out on a man known to have had achlorhydria for some time previously. No organic lesion of the stomach was present. A fractional test meal revealed entire absence of both free and combined hydrochloric acid. The chloride secretion was, however, perfectly normal. A fair amount of trypsin was present in the resting juice, but in subsequent samples the amounts were quite insignificant.

When this patient was given sodium hydrogen sulphate the acidity of the stomach contents, after a preliminary delay, fell rapidly until in one hour after taking the meal the fluid removed from the stomach was actually faintly alkaline. There was only a very slight change in the ratio of acid to total sulphate, but a marked fall in sulphate. Many other

experiments carried out gave similar results and showed very clearly that, when mineral acid is introduced into the stomach, it is possible for the acidity to be reduced and the stomach emptied with no evidence whatever that neutralisation plays any appreciable part in the reduction of the acid. It is highly probable that the diluting fluid brings neutral chlorides into the stomach, but this point will be discussed later.

*Experiments with a test meal containing sodium sulphate.* When sodium sulphate is dissolved in an ordinary wheat test meal in such an amount as to make the concentration of  $\text{SO}_4''$  about 0.1 normal (5 gm. "anhydrous"  $\text{Na}_2\text{SO}_4$  in 560 c.c. fluid) and the fractions removed after the meal are examined quantitatively for sulphate, it is in general found that the concentration of sulphate shows a progressive decrease until in about one and a half hours it has entirely disappeared from the stomach. In connection with these experiments we discovered a relationship between the amount of sulphate and the concentration of chloride in the fractions. The sum of the equivalent concentrations of  $\text{SO}_4''$  and  $\text{Cl}'$  proved to be remarkably constant throughout the interval between the ingestion of the meal and complete evacuation of the stomach.

The results of one of these experiments are given in Table IV. Here the initial concentration of sulphate in the meal was roughly 0.12 normal, from which it gradually fell until in one and a half hours nothing but traces of sulphate remained in the stomach.

TABLE IV. Normal subject.

Meal: Five grams of "anhydrous"  $\text{Na}_2\text{SO}_4$  dissolved in 560 c.c. of wheat test meal.  
 $\text{SO}_4 = 1.16 \text{ N}/10 = 0.824 \text{ p.c. Na}_2\text{SO}_4$ .

Note the constancy of the sum of  $\text{SO}_4''$  and  $\text{Cl}'$  in the fluid removed.

Time	Bile	Mucus	Total (Cl)' N/10	Neutral (Cl)' N/10	HCl N/10	( $\text{SO}_4$ )'' N/10	$\text{Na}_2\text{SO}_4$ gm. 100 c.c.	( $\text{SO}_4$ )'' + (Cl)' N/10
Resting juice:								
(13 c.c.)	trace	—	1.250	0.553	0.697	—	—	1.25
15 min.	—	—	0.213	0.066	0.147	0.968	0.687	1.18
30 "	—	—	0.325	0.075	0.250	0.860	0.611	1.19
45 "	—	—	0.722	0.169	0.553	0.526	0.373	1.25
60 "	—	—	0.853	0.111	0.742	0.376	0.267	1.23
75 "	—	—	1.030	0.133	0.897	0.202	0.143	1.23
90 "	—	—	1.250	0.200	1.050	0.010	0.071	1.26
103 "	—	—	1.270	0.270	1.000	—	—	1.27
120 "	—	—	1.190	0.585	0.605	—	—	1.19
140 "	—	—	1.060	0.508	0.252	—	—	1.06
170 "	trace	—	1.030	0.487	0.543	—	—	1.03
Average value = 1.19								

The sum of the equivalent concentrations of  $\text{SO}_4''$  and  $\text{Cl}'$  had an average value throughout the experiment of 0.12 normal.

A number of similar experiments were carried out on normal subjects, and the results were so constant as to satisfy us that this relationship is not a chance one, but depends upon a peculiar property of the gastric mechanism.

If we suppose that regurgitation of duodenal contents does not occur, and that the dilution of the meal, as shown by the falling sulphate, is brought about mainly by a mixture with gastric juice, this secretion must be the medium by which chloride is brought into the stomach.

If the stomach contains sulphate solution of about 0.11 normal strength (with no chloride) the only way in which chloride can be added to the stomach contents so as to keep the concentration of chloride plus sulphate at 0.11 normal is by means of a solution of 0.11 normal chloride. If any of the mixture escape from the stomach the sum would still remain constant.

We, therefore, regard these experiments as evidence that the gastric secretion under normal conditions has always approximately the same concentration of Cl' equal in strength to about 0.11 normal. This value corresponds roughly with the amount of Cl' in the plasma (0.095-0.106 normal).

It was in general found that the acidity commenced to fall, or was already falling, at the time of disappearance of sulphate from the stomach. At this point the stomach was practically empty.

In some experiments in which we used sulphuric acid instead of sodium sulphate similar results to those with sulphate were obtained.

#### DISCUSSION.

In this paper we have endeavoured to show that the fall in acidity which occurs towards the end of normal gastric activity cannot be explained adequately on the basis of duodenal regurgitation. This reduction in gastric acidity has been shown to occur without any evidence of the simultaneous appearance of trypsin and carbon dioxide, which one would expect if a reflux of duodenal fluid had taken place.

When acid is introduced into the stomach and the acidity of the contents is found subsequently to fall (Hicks and Visser<sup>(7)</sup> and Crohn<sup>(8)</sup>) the evidence we have adduced leads us to the conclusion that the acidity of the fluid introduced is lowered by the secretion of a neutral fluid containing neutral chloride.

We consider that a similar process occurs in the later stages of digestion when the concentration of acid diminishes.

Apperly<sup>(9)</sup> has described the gradual attainment of a definite Cl'

concentration by the gastric contents after a test meal; he calls this the "chloride point." Its value is equal approximately to the concentration of chloride in the blood plasma, and he has shown that the juice in the resting stomach has a similar value. Our results are in agreement with his findings; we believe that the gastric glands secrete at all times, whether active or quiescent, a fluid having a fixed concentration of chloride ion.

Since sodium chloride is found in the stomach in the absence of duodenal regurgitation, it must be secreted by the gastric glands. Gamble and Ross(10) have described how after ligature of the pylorus in dogs the stomach secretion contains large amounts of sodium chloride. Gamble and McIver(11) proved that the same phenomenon can occur in rabbits and remark that "the entrance into the stomach of a large amount of fixed base, more than three-quarters of the equivalence of  $\text{Cl}'$  loss, constitutes a finding which is probably of important significance as regards the construction and function of gastric juice." Later the same workers(12) demonstrated, on cats with gastric pouches, that in the presence of a fairly constant value for  $\text{Cl}'$  quite large variations in fixed base could occur; these variations seemed to depend on the nature of the meal.

Pavlov(13) believed that the acidity of the gastric juice is constant, although he observed that the first portions of the juice secreted from a gastric pouch were often less acid than subsequent samples; he ascribed this to partial neutralisation of the juice by mucus on the wall of the resting stomach, an effect which passed off in a short time. On the other hand, Rosemann(14), in similar experiments, observed that the change occurred both at the beginning and the end of secretory activity, while the total chlorine concentration remained fairly constant, and Katsch(15) holds that one must accept a variation in the hydrochloric acid of the gastric juice.

There is, therefore, already evidence that (1) the gastric glands can secrete sodium chloride, and (2) that the concentration of this salt undergoes change. Since, as we have shown, duodenal regurgitation is not the prime factor in the regulation of gastric acidity, we have put forward evidence for the view that the chloride ion brought to the glands by the blood as sodium chloride is secreted at a definite fixed concentration, part of it unchanged as sodium chloride and part changed into hydrochloric acid, and that the extent of this change governs the acidity of the secreted juice. Thus during the early stages of digestion, when the acidity of the gastric contents is rising, there is a marked change of sodium chloride into hydro-

chloric acid, so that the neutral chloride curve is low; but when the acidity reaches a certain value, which may be different in different subjects, the degree of transformation of sodium chloride diminishes until more or less all this salt is secreted unchanged. This brings about a fall in acidity and a corresponding rise in neutral chloride as shown by the gastric analysis curves.

Finally, when the stomach is empty, the rate of secretion falls to the fasting level and the juice takes on the character of the resting secretion, which is only slightly acid.

### CONCLUSIONS.

(1) The fall in concentration of hydrochloric acid and corresponding rise of sodium chloride in the stomach contents during digestion are not brought about by regurgitation of alkaline fluid from the duodenum, as is generally supposed; slight regurgitation of alkaline fluid into the stomach occasionally takes place, but this plays little or no part in the regulation of gastric acidity.

(2) The normal stomach secretes the chloride ion in about the concentration in which it is present in the blood. Some of the  $\text{Cl}'$  is secreted as hydrochloric acid while the remainder is secreted as neutral chloride, the proportion of the two forms being different at different stages of digestion.

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# VARIATIONS IN THE ACIDITY AND TOTAL CHLORIDE CONTAINED IN THE SECRETION FROM AN ISOLATED PAVLOV POUCH IN THE DOG.

BY HUGH MACLEAN, WILLIAM J. GRIFFITHS  
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IN the foregoing paper two of us described experiments on the human subject the results of which are capable of being put to the test in dogs with an isolated gastric pouch as planned and used by Pavlov(1). If the secretion from such a pouch shows a fluctuating acidity but a constant concentration of total chloride, such as we described and studied in the paper referred to, there is no possibility that decline of acidity can be due to regurgitation of intestinal contents into the pouch. Pavlov's operation was accordingly performed on two dogs, and ample time was allowed for them to recover from the effects. By inserting a cannula into the fistula the secretion could be collected in tubes, the dog being maintained in the upright position. The tubes were changed when secretion sufficient for analysis had collected. In this way a fractional analysis of the secretion from the pouch during gastric digestion could be carried out. The samples were analysed for total chloride and neutral chloride (after careful ignition to drive off the hydrochloric acid), the value for hydrochloric acid being obtained by difference. For the determination of chlorine the method of Van Slyke(2) was employed because it gives accurate results with samples of 1 c.c. or less. The samples obtained were water clear except for occasional flecks of mucus. The integrity of the pouches was established post-mortem.

We found that the value for total chlorine in the secreted juice remained remarkably constant throughout long periods of observation. Typical figures are recorded in Table I. Notwithstanding this constancy of the total chlorine, the acidity of the juice usually underwent marked

TABLE I.

Dog No. 1	1.56	1.67	1.69	1.65	1.71	1.65	1.65	1.64	1.68
Dog No. 2	1.47	1.51	1.53	1.49	1.51	1.50	1.49	1.53	—
Dog No 2	1.51	1.49	1.48	1.50	1.49	1.53	—	—	—

Showing constancy of the concentration of total Cl' (N/10) in juice secreted at varying intervals after feeding.



changes, dependent on the stages of digestion. Immediately after feeding, the acidity of the juice was found to rise fairly rapidly to a high level to be followed by a sharp fall, sometimes at once, sometimes after a varying interval of time.

The analytical figures for each sample clearly represent the *average* composition of the juice over the period of collection; for this reason, and because of a rather slow rate of secretion, the actual concentration of acid at the beginning of the period after feeding, as well as the minimum value reached during the decline in acidity, could not be accurately determined, but they must obviously be lower than those recorded. We know that the secretion of the glands during the intervals between active digestion is neutral, or only very slightly acid in reaction.

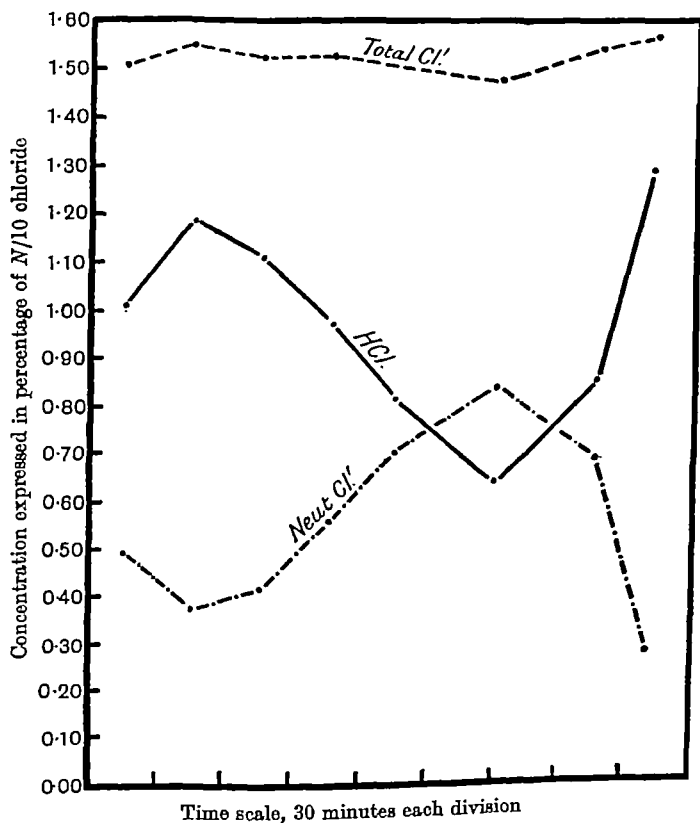


Fig. 1. Curves obtained from the secretion of an isolated gastric pouch in dog No. 1.

When the stimulus of the first meal had passed off, and the acidity and rate of secretion of the juice had fallen, a second meal was given. There was an immediate response, the acidity rising again; these fluctuations occurred with little or no change in the total chlorine concentration, and because of this, the amount of chlorine which is bound to basic ions follows a path exactly opposite to that of the acidity. The concentration of chlorine in the juice being fixed, a diminution in hydrochloric acid must be accompanied by an increase in neutral salts.

Figs. 1 and 2 represent some results obtained from the secretion of

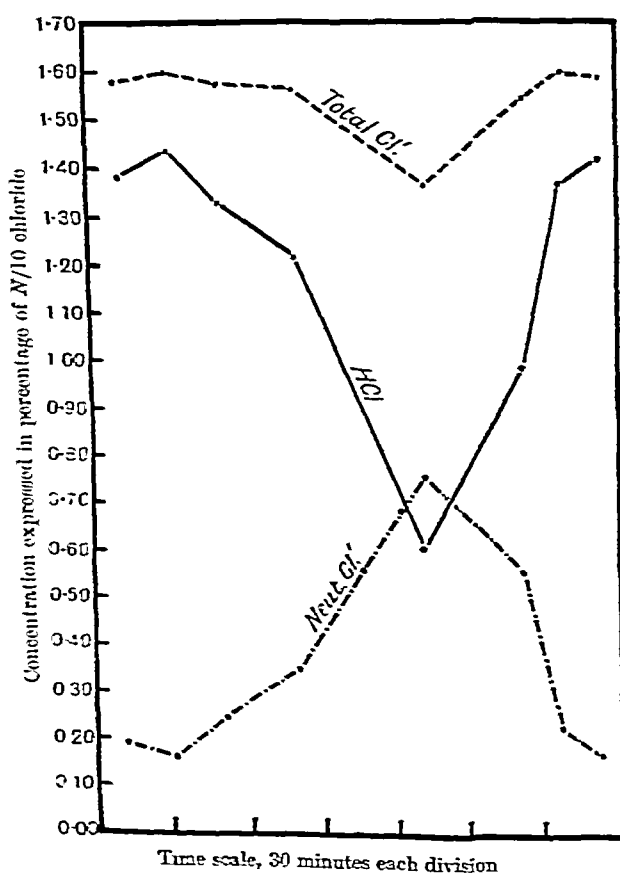


Fig. 2. Curves obtained from secretion of gastric pouch in dog No. 2.

these pouches. In Fig. 1 the dog was given 100 grm. minced flesh and the first sample of secretion from the pouch was obtained 30 min. after-

wards. By this time the hydrochloric acid concentration had reached 1.01 *N*/10. In the next sample collected during the next half-hour the concentration had risen to 1.18 *N*/10. After this the acid began to fall until it reached the level of 0.63 *N*/10 when another meal consisting of 100 gm. of minced meat was given. From this point the acid of the pouch began to rise until it reached a concentration of 1.29 *N*/10, at which point the experiment was stopped. It is interesting to note how the sodium chloride concentration varied exactly in inverse ratio to the acid concentration during the whole of this experiment.

In Fig. 2 similar results obtained from another dog are shown. The meal given in this case was 40 gm. of dog biscuit soaked in water. This meal was repeated after the hydrochloric acid had fallen to a concentration of 0.61 *N*/10. After this, the acid concentration increased to a maximum of 1.42 *N*/10 at which point collection of specimens was stopped. Here, again, the total chlorine remained fairly constant, while the sodium chloride varied in inverse proportion to the hydrochloric acid.

As the result of various other experiments on these dogs it was found that the secretion of gastric juice in the pouch followed the general secretion in the stomach. The comparatively high acid concentration was reduced as digestion in the stomach proceeded, until a practically acid-free fluid was obtained. For reasons already explained it was difficult to get sufficient fluid for quantitative analysis at this stage, but qualitative tests show that juice secreted at this period had only a very faintly acid reaction. There was obviously no possibility of regurgitation of alkaline duodenal fluid into the pouch, yet the contents of the pouch showed a fall of acid with a corresponding increase of neutral chloride, just as is found during ordinary gastric digestion. This seems to prove conclusively that regurgitation is not the cause of the fall of acidity in the course of ordinary digestion.

#### DISCUSSION.

It is clear from the results outlined above, that in dogs the acidity of the gastric secretion, as collected from the Pavlov pouch, is different at different intervals of time after meals; the total chlorine content of the juice, however, is maintained at a fixed level. An increase in acid is accompanied by a decrease in neutral chloride, and *vice versa*.

The same constancy of total chloride ion concentration with varying concentrations of hydrochloric acid and sodium chloride may be observed in man. Fig. 3 shows the composition of the fluid removed at intervals from a normal human stomach following a drink of 300 c.c.

of 5 p.c. alcohol. A solution of alcohol of this strength stimulates gastric secretion, and the secretion is continued long after the alcohol has left

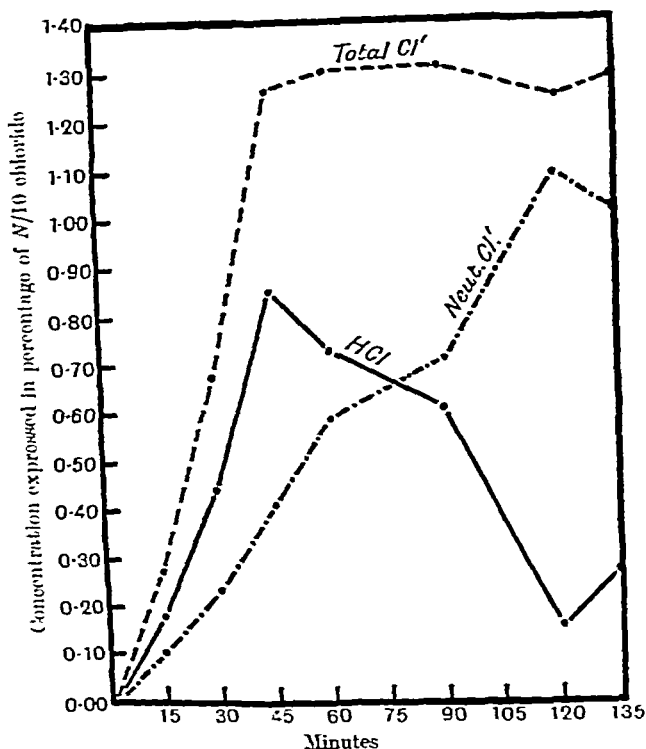


Fig. 3. Curves obtained from a normal subject after test meal containing 5 p.c. alcohol.

the stomach, so that within half an hour the fluid removed from the stomach is practically pure gastric juice. The samples were colourless and water clear, and presumably no regurgitation had occurred. It will be seen that the total chlorine rapidly attained a value of about 1.3 N/10, and that this value was maintained for 1½ hr., when no further juice could be obtained from the stomach; during this time the acidity showed very marked changes in a manner exactly similar to that observed in the juice secreted by the gastric pouches in the dog.

Pavlov was firmly of the opinion that the juice as poured out by the gastric glands in the dog had a constant acidity. Rosemann(3) and others have since denied that this is true. Rosemann's investigation of the chemical composition of the gastric secretion yielded results similar to those described above, but as his experiments were carried out by the

method of "sham feeding," and the connection between the stomach and duodenum was not interfered with, the possibility of duodenal regurgitation was not eliminated.

The sodium chloride present in the gastric secretion of the dogs used by us must have been secreted by the glands situated in the pouch. This salt can no longer be regarded merely as an index of the extent of neutralisation of acid by regurgitation of duodenal alkali into the stomach.

As to the mechanism which controls the conversion of chloride to hydrochloric acid, it is interesting to note that there is evidence that the presence of acid in the stomach exerts, as it were, a "back-pressure," in such a way as to tend to drive back or suppress the further production of acid by the gastric glands. Vandorfy(4) showed that if acetic acid of sufficient concentration is introduced into the human stomach it inhibits the secretion of hydrochloric acid. We observed the same phenomenon using both acetic and hydrochloric acids before we were aware of Vandorfy's work. Although the production of hydrochloric acid is stopped, secretion of neutral chloride still goes on.

#### CONCLUSIONS.

(1) The secretion obtained from an isolated pouch of the dog's stomach during digestion behaves with regard to hydrochloric acid and sodium chloride exactly as does the secretion of the normal human stomach. There is first a rise in acid concentration, followed by a definite fall; when digestion is over this juice contains practically no hydrochloric acid.

(2) The total chloride present in this pouch secretion remains approximately constant during the whole period of gastric digestion.

(3) Since the acid concentration in an isolated pouch into which no regurgitation of alkaline duodenal fluid can possibly take place shows, during digestion, a reduction of acid and a corresponding increase of sodium chloride in the same manner as is found in the normal stomach, it follows that regurgitation is not necessary to bring about the reduced acidity found in the normal stomach during the later stages of digestion. This reduction is brought about by the stomach quite irrespective of any regurgitation that may take place.

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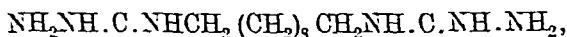
# THE RELATION OF SYNTHALIN TO CARBOHYDRATE METABOLISM.

By R. BODO AND H. P. MARKS.

*(From the National Institute for Medical Research, Hampstead,  
London, N.W. 3.)*

## INTRODUCTION.

Noel Paton, Watanabe<sup>(1)</sup> and others have observed that the intoxication following administration of guanidine is accompanied by hypoglycæmia, and Frank, Nothmann and Wagner<sup>(2)</sup> have recently made an extensive study of guanidine derivatives, with the object of trying to find a substance with a more pronounced hypoglycæmic action, but lower toxicity. As a result, they have synthesised a guanidine derivative which is now being produced commercially as a substitute for insulin, under the name of synthalin. Frank has given the composition of synthalin as decamethylene-diguanidine, which has the following structural formula:



the substance issued for therapeutic use being the dihydrochloride of this base. We used in our earlier experiments the tablets issued for oral administration, but more recently we have obtained a supply of the pure salt through the courtesy of Messrs Schering, Ltd. This substance is claimed to have an insulin-like action. It is said to differ from insulin, however, in two important respects. Firstly, it is effective when given by the mouth, and, secondly, the action is delayed, *i.e.*, the blood sugar may remain quite normal for several hours before the hypoglycæmia develops.

## I. EXPERIMENTS ON WHOLE ANIMALS.

While not concerning ourselves with the oral administration of synthalin, we considered it desirable to study the effect of parenteral injections on the blood sugar of the whole animal. At first, injections of from 1 to 5 mg. were given to rabbits (previously fasting for 24 hr.), on the assumption, made by Frank, that 1 mg. of synthalin corresponds roughly to 1 unit of insulin, but without observable effect on the blood

method of "sham feeding," and the connection between the stomach and duodenum was not interfered with, the possibility of duodenal regurgitation was not eliminated.

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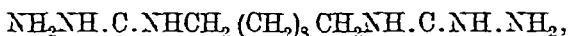
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sugar. Using larger doses, however, we were able later to reproduce the delayed hypoglycæmia described by Frank. The following are a few typical experiments:

*Exp. 1.* Rabbit weighing 2.3 kilos received 20 mg. synthalin subcutaneously.

Hours after injection	0	1	2	3	4	5	6	7
Blood sugar in mg. p.c.	113	125	116	130	95	79	79	63

Died later in hypoglycæmic collapse. Liver glycogen 0.03 p.c.

*Exp. 2.* Rabbit weighing 2.3 kilos, 50 mg. synthalin.

Hours after injection	0	1	2	3	4	5
Blood sugar in mg. p.c.	139	294	260	164	96	72

Died at fifth hour. Liver glycogen 0.05 p.c.

*Exp. 3.* Rabbit weighing 2.0 kilos, 50 mg. synthalin.

Hours after injection	0	1	2
Blood sugar in mg. p.c.	135	118	71

Died at second hour. Liver glycogen 0.02 p.c.

*Exp. 4.* Rabbit weighing 2.6 kilos, 7 mg. synthalin.

Hours after injection	0	1	2	3	4	5	6
Blood sugar in mg. p.c.	106	125	113	117	122	120	119

No symptoms.

*Exp. 5.* Rabbit weighing 2.9 kilos, 20 mg. synthalin.

Hours after injection	0	1	2	3	4	5	6
Blood sugar in mg. p.c.	147	179	194	251	251	209	267

No symptoms, complete recovery.

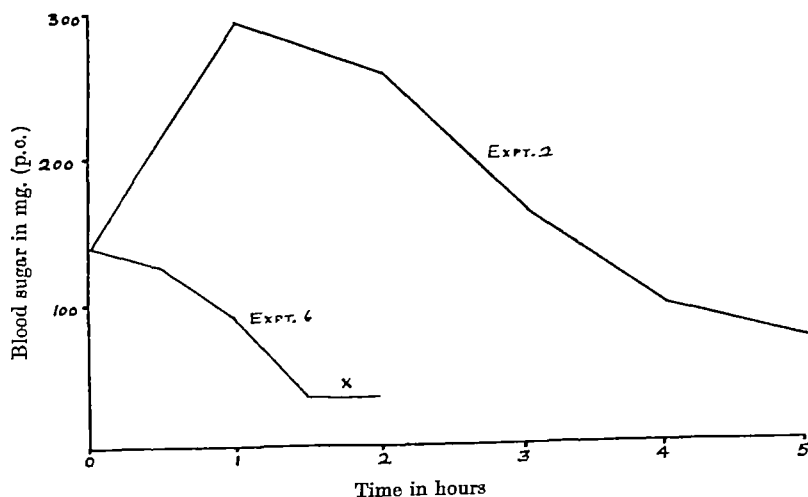


Fig. 1. (Exps. 2 and 6). *Effect of synthalin on blood sugar.* 50 mg. synthalin injected at 0.

Exp. 2 shows hyperglycæmia followed by hypoglycæmia.

Exp. 6 shows immediate hypoglycæmia, with convulsions at X.

In these experiments the synthalin, obtained in tablet form, was injected subcutaneously in saline solution. Fig. 1 (Exp. 6) illustrates the effect of injecting 50 mg. of pure synthalin.

Exps. 1 and 2 are typical of the effect produced by 10–15 mg. of synthalin in rabbits fasted for 24 hours previously, namely, a period of normal or raised blood sugar level, followed by a marked hypoglycæmia which usually terminates fatally, unless glucose is promptly given.

As already mentioned, we were, in general, unable to obtain any effect with the smaller doses advocated by Frank (Exp. 4), while in rare cases, even large doses produced the anomalous effect shown in Exp. 5. On the other hand, increasing the dose usually accelerated the onset of hypoglycæmia, as in Exp. 3 (and Exp. 6, Fig. 1).

The very low figures for liver glycogen deserve special consideration, as they suggest a possible difference between the actions of synthalin and of insulin respectively. For insulin does not normally reduce the liver glycogen to such low levels, unless severe hypoglycæmic convulsions supervene. In order to demonstrate this difference, a rabbit, which had fasted for 24 hours previously, as in the case of the synthalin experiments, was given half a unit of insulin subcutaneously at hourly intervals, so as to produce a hypoglycæmia lasting over several hours. Actually, the blood sugar level fell to 0.05 p.c. in 2 hours and remained below 0.08 p.c. until the ninth hour after the first injection, when the animal was killed with a blood sugar of 0.09 p.c. The liver was found to contain 0.5 p.c. of glycogen. This is within the normal range, as is shown by the following figures for four similar rabbits which were simply kept without food for 24 hours and then killed: 0.42, 1.5, 0.25, and 1.7 p.c.

In the above experiments, where the liver glycogen was studied in the hypoglycæmic phase of synthalin action, a depletion was regularly observed, but animals killed in the hyperglycæmic phase illustrated in Exps. 2 and 5 usually had still some liver glycogen left. Thus, a rabbit, killed 1 hour after receiving 70 mg. of synthalin, with a blood sugar of 0.190 p.c. was found to have 0.3 p.c. of glycogen in the liver, while another rabbit, killed 2 hours after receiving 20 mg. of synthalin, with a blood sugar of 0.215 p.c. had a liver glycogen of 0.1 p.c. Quite recently both Staub<sup>(3)</sup> and Arndt<sup>(4)</sup> have published observations on blood sugar changes and discharge of glycogen similar to those presented above.

This action of synthalin in depleting the liver of glycogen suggested an investigation of the effect of adrenaline on the blood sugar of an animal previously injected with synthalin.

In Fig. 2 the effect of injecting subcutaneously 0.5 mg. of adrenaline

into a rabbit injected with synthalin several hours previously is compared with the effect of the same dose of adrenaline on a rabbit maintained in a hypoglycæmic condition by hourly injections of half a unit

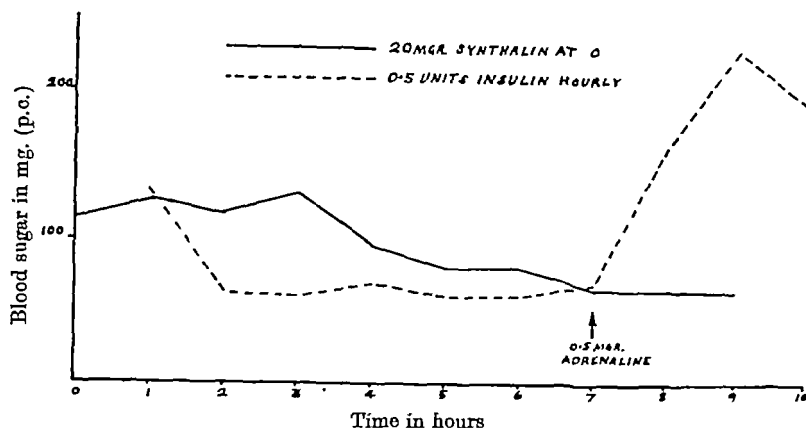


Fig. 2 (Exp. 7). Abolition of adrenaline response.

of insulin. The curves demonstrate in a striking manner the complete absence of the usual hyperglycæmic response to adrenaline in the animals previously treated with synthalin. This absence of response cannot be attributed to the occurrence of hypoglycæmia, which in this experiment was slight, since the animals in which a prolonged and more pronounced hypoglycæmia was induced by insulin gave a definite response to the injection of adrenaline.

This behaviour towards adrenaline was regularly observed in our experiments, and only a few typical protocols will be given.

*Exp. 8.* Rabbit weighing 2.9 kilos received 20 mg. synthalin. 0.5 mg. adrenaline injected at \*, 4 hours later.

Hours after injection	0	1	2	3	4*	5	6
Blood sugar in mg. p.c.	116	86	129	107	100	94	107

Killed at sixth hour. Liver glycogen 0.08 p.c.

*Exp. 9.* Rabbit weighing 2.9 kilos received 20 mg. synthalin. 0.5 mg. adrenaline injected at \*, 2 hours later.

Hours after injection	0	1	2*	3	4	5
Blood sugar in mg. p.c.	90	116	125	72	45	54

Convulsed at fifth hour. Liver glycogen 0.04 p.c.

*Exp. 10.* Rabbit weighing 2.0 kilos received 25 mg. synthalin. 0.5 mg. adrenaline injected at \*, 1 hour later.

Hours after injection	0	1*	2	3
Blood sugar in mg. p.c.	95	143	239	58

Killed at third hour. Liver glycogen 0.06 p.c.

## SYNTHALIN AND CARBOHYDRATE METABOLISM. 87

*Exp. 11.* Rabbit weighing 2.5 kilos received 15 mg. synthalin. 0.1 mg. adrenaline injected at \*, 1 hour later.

Hours after injection	0	1*	2	3	4	5	6
Blood sugar in mg. p.c.	114	132	168	166	190	166	162

*Exp. 12.* Rabbit weighing 3.0 kilos received 15 mg. synthalin. 0.5 mg. adrenaline injected at \*, 3 hours later.

Hours after injection	0	1	2	3*	4-8	9
Blood sugar in mg. p.c.	123	103	113	145	> 350	330

Killed three months later. Liver glycogen 4.2 p.c.

In cases where synthalin by itself does not appreciably raise the blood sugar the latter is unaffected by the injection of adrenaline, as in Exps. 7 and 8. Where, however, the synthalin itself produces a rise in blood sugar, this rise may either give place to a fall as in Exp. 9, or may continue its course, especially when the adrenaline is injected shortly after the synthalin, as in Exps. 10 and 11. Thus, in Exp. 10, the blood sugar continues to rise for 1 hour after the injection of adrenaline, and then rapidly falls, while in Exp. 11 the rise continues for several hours. We feel justified in attributing the continued rise to the synthalin, and not to the adrenaline, since, with the possible exception of Exp. 12, it is of quite a different order from that produced by the same dose of adrenaline in a normal animal. We usually observed the blood sugar to rise to between 0.3 p.c. and 0.4 p.c. within 3 hours after the injection of 0.5 mg. of adrenaline into a normal fasting rabbit.

Exp. 12 deserves special mention on account of the considerable hyperglycæmia observed. This experiment was performed on the same animal as had shown such an extensive hyperglycæmia after the injection of synthalin in Exp. 5. This animal, therefore, reacted very strongly towards synthalin with a discharge of liver glycogen, which we may reasonably attribute to the presence of an abnormally large glycogen reserve in the liver; for, three months later, the liver was found to contain 4.2 p.c. of glycogen after a 24 hours' fast.

In general, we would suggest that the behaviour of the blood sugar after an injection of synthalin depends upon the amount of the glycogen reserves, for we regularly find more glycogen present in the livers of rabbits killed in the hyperglycæmic phase than in those killed with a low or falling blood sugar.

A similar view is taken by Staub(3), who further claims to have eliminated the initial hyperglycæmia by the previous administration of ergotamine.

It would seem, therefore, that synthalin interferes in some way with the maintenance of the glycogen reserves in the liver, and that when

the reserves already present are exhausted, the blood sugar falls. This hypoglycæmia may be either immediate or delayed, according to the dose of synthalin given and the state of the glycogen reserves.

That synthalin may have a wider and perhaps toxic action on the liver is suggested by Morawitz<sup>(5)</sup> and Adler<sup>(6)</sup>, who observed a development of jaundice after treatment with synthalin, and by Bertram<sup>(7)</sup>, who reports the development of acute yellow dystrophy of the liver in a patient treated with synthalin. Also the use of "decholin" (sodium dehydrocholate) in order to alleviate the unpleasant symptoms sometimes produced by synthalin suggests that these symptoms may be the expression of an impairment of liver function. This view is taken by Szczeklik<sup>(8)</sup>, who also observed the development of jaundice in one of his patients. Hornung<sup>(9)</sup> has observed a derangement of liver function, as indicated by the phenol-tetrachlorphthalein test.

With regard to histological evidence of liver damage Simola<sup>(10)</sup> notes the occurrence of fatty degeneration in animals dying two or three days after administration of synthalin, and Staub<sup>(3)</sup> and Hornung<sup>(9)</sup> in animals to which small doses of synthalin were administered daily for several weeks. Arndt<sup>(4)</sup>, however, could observe in such cases no alteration beyond an exhaustion of the glycogen reserves. We have not so far investigated the histological changes occurring in the liver after such prolonged subjection to the action of synthalin, but Dr P. P. Laidlaw, F.R.S., has kindly examined the livers of several rabbits used in acute experiments and we are indebted to him for the following report:

"Sections from the livers of seven rabbits were submitted for microscopic examination. Very varying degrees of abnormality were found from case to case. In one instance there was striking hydropic degeneration of all the cells at the periphery of the liver lobules, involving more than half of the whole area examined, together with a few small areas of complete necrosis. There was very little fat in the cells of the degenerated area and leucocytes were present in abnormal numbers between the degenerating cells. In other instances only small isolated patches of this same type of degeneration could be found in its advanced form, but the appearances at the periphery of the lobule suggested that the process was developing over a considerable area. In one case, where synthalin had been given on three successive days, there was extensive and severe fatty degeneration at the periphery of the lobule, gradually diminishing towards the central vein.

"The impression left from the examination of all the specimens is that

synthalin may cause severe and widespread injury to the liver parenchyma, up to and including death of the liver cells. The injury is maximal at the periphery of the lobule and may cause an unusual type of liver degeneration. The paucity of the material and the varying conditions to which the experimental animals were submitted do not allow of any firm conclusion being made. The matter is one which appears to merit further attention and more extensive observations will be made."

A toxic action on the liver, interfering with its glycogenic function, and probably depressing the new formation of carbohydrates, might obviously by itself lead to a progressive hypoglycæmia, as soon as the glycogen reserves of the liver had been exhausted. Such an effect might be expected to produce the commonly observed sequence of hyperglycæmia followed after a few hours by progressive hypoglycæmia. The foregoing experiments give good reason for believing that an important part of the action of synthalin could be accounted for on these lines. There remained the question whether such an action could account for the whole of its effect, or whether it resembled insulin in causing an accelerated disappearance from the circulation of glucose preformed as such. In the latter case it should be possible to demonstrate the effect of synthalin, like that of insulin, on the blood sugar of the spinal, eviscerated cat<sup>(11)</sup> and on the perfused limb<sup>(12)</sup>.

## II. EXPERIMENTS ON THE EVISCERATED SPINAL ANIMAL AND ON PERFUSED MUSCLE PREPARATIONS.

*Methods.* In this preparation<sup>(11)</sup> the cord is cut under ether anaesthesia, after previously tying both carotids, and, as there is still a blood supply to the brain by way of the vertebrals, the animal is further pithed, and the foramen magnum plugged with a cork. In spite of this there is usually some loss of blood, and the animal rarely survives the subsequent evisceration for more than 3 or 4 hours. In dealing with insulin this is of little consequence, since the effect of insulin on the rate of sugar disappearance is so marked as to be demonstrable beyond question in a short time, whereas in our earlier experiments we found it impossible to establish an incontestable effect of synthalin without extending the experiment for many hours. This was largely due to the fact that at that time synthalin was available to us only in the form of tablets containing a large amount of binding material (presumably containing lactose). This introduced two difficulties. Firstly, the binding material was found to have such a high reducing power that its injection into the blood stream might well mask any slight fall in blood sugar caused by the synthalin.

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Secondly, the preparation was so toxic on intravenous injection that it was found impossible to give more than the equivalent of 20 mg. of the synthalin without killing the animal. More recently, we have obtained a sample of pure synthalin, through the courtesy of Messrs Schering, up to 100 mg. of which may sometimes be administered to cats prepared by an improved method. It seems probable, therefore, that the toxicity of the original preparation, when given intravenously, was largely due to the binding material which it contained.

The chief improvement in technique consists in cutting off completely the blood supply to the head and neck by tying, in addition to the carotids, the vertebral, the costo-cervical and the thyroid axis arteries on both sides. After this the brain may be pithed without loss of blood, and a preparation obtained which will last for 10-12 hours.

In short, the method of preparation is as follows: Under ether, preceded by chloroform, anaesthesia, both carotids are first tied in the neck, and both vagi prepared for later division. The upper end of the sternum is then divided, the subclavians exposed without damaging the pleura, and both vertebrales, costo-cervical axes and thyroid axes ligatured close to their junction with the subclavians. The latter, together with the mammary arteries, are left intact.

At this point it should be mentioned that, whereas in the cat, the right vertebral leaves the subclavian opposite to the mammary, on the left side the origin is much deeper. The tying of these vessels requires some care, but after some practice the operation can easily be performed. The surest indication that the right vessels have been tied is the sudden cessation of natural respiration, after which the artificial respiration is immediately started, the vagi cut and the brain pithed. Then the evisceration and removal of the kidneys are carried out in the manner previously described by Burn and Dale.

In some of our experiments we used the isolated hind leg preparation from the dog. The hind legs were perfused by the method described by Best<sup>(12)</sup> on cats, with the difference that the lungs were used to oxygenate the blood, instead of the artificial oxygenator. The double perfusion pump described by Dale and Schuster<sup>(13)</sup> was used, the dog being chosen for these experiments in order to obtain a convenient volume of blood.

In those experiments on the eviscerated, spinal cat where it was necessary to measure the respiratory metabolism, this was done with the Schuster closed circuit spirometer by the method described in a previous paper from this Institute<sup>(14)</sup>.

*Experiments on eviscerated. spinal cats.*

As a result of the anaesthesia and the operative technique, the preparation usually starts with a high blood sugar, the level of which is allowed to fall to about 0.2 p.c. before commencing the glucose infusion. Even then the results are often vitiated by the irregular drainage into the circulation of sugar from the liver, whose circulation is only interrupted on the inflow side, so that it is necessary to prolong the initial control period of sugar infusion, in order to be quite sure that the blood sugar has really arrived at a final steady level, before injecting the synthalin. In one of our earlier control experiments, after first obtaining an apparent sugar balance, we observed a spontaneous fall in the blood sugar which corresponded almost exactly, over a period of several hours, to the fall observed in another experiment after injection of synthalin. In the case of experiments on insulin such spontaneous changes were of no account, since the effect of insulin was immediate and the fall of blood sugar following its injection much more rapid than any seen without it. In the case of synthalin the delayed onset of the relatively slow action demanded much greater precautions. A number of such experiments forced us to the conclusion that the only way to establish an effect of synthalin was to prolong the control period until we were sure that a permanent balance, preferably with a slowly rising blood sugar, had been obtained. This means that, in a fasting cat, synthalin cannot safely be given until 5 or 6 hours after the evisceration. But it will be seen from the following protocols, and from Fig. 3, that, given

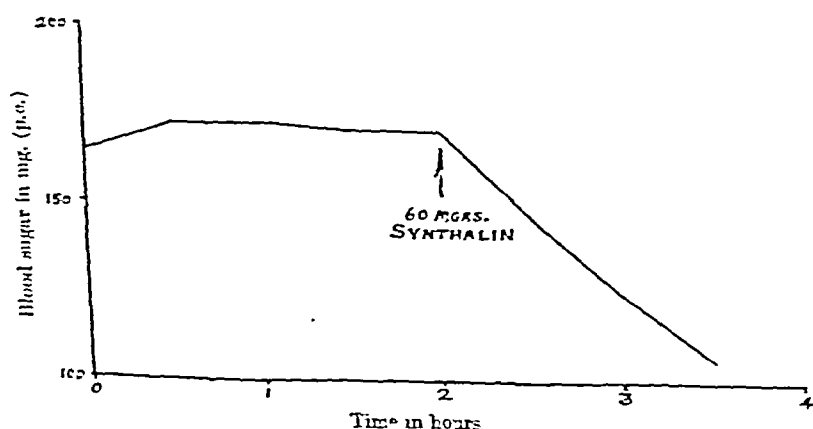


Fig. 3 Increased disappearance of glucose in the eviscerated, spinal cat, with continuous infusion of glucose.

a strong, healthy cat which will stand a large dose of synthalin, the effect of the latter on the blood sugar is beyond question.

*Exp. 13.* Eviscerated, spinal cat. Weight 2.9 kilos. Infusion of 4 p.c. glucose at 4.8 c.c. per hour. 60 mg. pure synthalin injected at  $\uparrow$ .

Time	5.0	5.20	5.40	6.0	6.5-6.10	6.40	7.10	7.40
Blood sugar	284	278	274	274	$\uparrow$	266	222	193
Time	8.10	8.40	9.10	9.40				
Blood sugar	155	124	119	138				

*Exp. 14.* Eviscerated, spinal cat. Infusion of 4 p.c. glucose at 10.2 c.c. per hour. 60 mg. pure synthalin injected at  $\uparrow$  and 100 mg. at  $\uparrow\uparrow$ .

Time	2.0	2.30	3.0	3.30	4.0	4.30	5.0	5.30	6.0
Blood sugar	197	175	190	195	181	191	200	$\uparrow$ 195	195
Time	6.30	7.0	7.30	8.0	8.30	9.0	9.30	10.0	
Blood sugar	211	189	182	$\uparrow\uparrow$ 155	157	139	153	139	

*Exp. 15.* Eviscerated, spinal cat. Weight 3.1 kilos. Infusion of 4 p.c. glucose at 7.2 c.c. per hour. 60 mg. pure synthalin injected at  $\uparrow$  and 50 mg. at  $\uparrow\uparrow$ .

Time	4.0	4.30	5.0	5.30	6.0	6.30	7.0	7.30	8.0
Blood sugar	232	243	248	248	$\uparrow$ 250	241	217	199	191
Time	8.30	9.0	9.30	10.0					
Blood sugar	$\uparrow\uparrow$ 172	157	145	162					

*Exp. 16.* See Fig. 3.

The above experiments demonstrate that large doses of synthalin have an accelerating effect on the disappearance of sugar from the blood of the spinal eviscerated preparation. Though much less pronounced than the effect of insulin, and needing special precautions for its detection, the effect is quite definite. There are, however, in the eviscerated, spinal preparation, at least three complicating factors which may to some extent give rise to a misleading result, namely: the drainage of glucose from the isolated liver, the occurrence of twitches or increased tone in the skeletal muscles through stimulation of the still intact spinal cord, and the direct effect of synthalin on the glucose consumption of the beating heart.

In order to eliminate all these factors, therefore, the action of synthalin on the isolated, perfused hind leg was investigated, this being the nearest approach to a pure skeletal muscle preparation.

#### *Experiments on the perfused hind leg.*

The two following experiments were performed on the isolated hind legs of the dog.

Exp. 17. Dog's hind legs and lungs perfused with defibrinated blood. Infusion of 4 p.c. glucose at 18 c.c. per hour. 50 mg. pure synthalin injected at ↑ and 25 mg. at ↑↑.

Time	1.20	1.40	2.0	2.20	2.50	3.20	3.50	4.20	4.50
			↑					↑↑	
Blood sugar	106	101	104	101	101	97	97	79	85
Time	5.20	5.50							
Blood sugar	92	124							

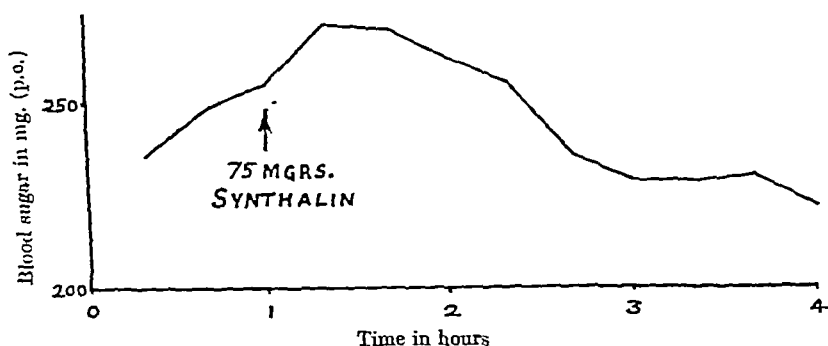


Fig. 4 (Exp. 18). Perfused hind legs of dog, with infusion of glucose.

After 50 mg. of synthalin the blood sugar fell from 104 to 79 mg. p.c. in 2 hours 20 minutes. After the second dose of synthalin a vasodilatation developed, which eventually absorbed the whole of the reserve of circulating blood. The rise of the blood sugar following it was no doubt due to failing vitality of the muscles with the inadequate circulation.

In the second experiment, shown in Fig. 4, in which the blood sugar was started at a higher level by a preliminary addition of glucose, before the regular infusion was started, the whole 75 mg. of synthalin was added at one time. In spite of the fact that the blood sugar was then slowly rising the addition of synthalin was followed by a fall from 255 to 228 mg. p.c. in the course of 2 hours.

We see, therefore, that in the skeletal muscle preparation, as represented by the isolated, perfused hind leg, synthalin still causes some increase in the rate of disappearance of glucose.

The question still remains as to what happens to the sugar which disappears. Is it stored as glycogen in the muscles, as in the case of insulin, or can it be accounted for by an increase in metabolism? In order to answer these questions further experiments were performed in which the changes in muscle glycogen content and in the respiratory metabolism were studied in the eviscerated, spinal cat preparation. Hoet(15) has already observed a loss of muscle glycogen in the eviscerated preparation after synthalin, a result which our experiments confirm, and Staub(3)

has recently reported similar experiments, in which he obtained results similar to ours, both as regards muscle glycogen and metabolism.

### *Muscle glycogen experiments.*

In these experiments no attempt was made to produce a fall in the blood sugar, but the latter was maintained at a high level by an increased infusion of glucose, so as to give the muscles every chance to deposit the excess of glucose as glycogen. In this, and in other respects, the technique was similar to that employed by Best, Hoet and Marks<sup>(16)</sup> in experiments with insulin.

*Exp. 19.* Eviscerated, spinal cat. With infusion of 4 p.c. glucose at 12 c.c. per hour, the blood sugar rose from 416 to 456 mg. p.c. during the three hours following the injection of 60 mg. of synthalin.

Muscle glycogen p.c.:

	Right leg before synthalin	Left leg 3 hours after synthalin
Quadriceps femoris	0.57	0.33
Biceps femoris	0.72	0.50
Gastrocnemius	0.52	0.33

*Exp. 20.* Eviscerated, spinal cat. With infusion of 4 p.c. glucose at 9-12 c.c. per hour, the blood sugar rose from 536 to 612 mg. p.c. during the 2 hours following the injection of 60 mg. of synthalin.

Muscle glycogen p.c.:

	Right leg before synthalin	Left leg 2 hours after synthalin
Tibialis anticus	0.68	0.33
Gastrocnemius	0.98	0.74
Sartorius	0.85	0.80

It has previously been observed<sup>(16)</sup> that the muscle glycogen in the eviscerated spinal preparation will remain sensibly constant over several hours, so that we may reasonably take the above experiments to indicate a breakdown of glycogen as a result of injecting synthalin.

At this point, therefore, the action of synthalin differs radically from that of insulin which, as we know, promotes the storage of glycogen by the muscles of the eviscerated, spinal preparation, especially where, as in the synthalin experiment, a persistent hyperglycæmia is maintained by accelerating the sugar infusion. We cannot, therefore, look to a conversion into glycogen to account for the extra sugar disappearing from the circulation under the action of synthalin.

The other obvious possibility is that the disappearing sugar may have been oxidised, in which case we should expect to observe a corresponding increase in respiratory oxygen consumption.

*Metabolism experiments.*

In the experiment illustrated by Fig. 5 the oxygen consumption of the preparation was determined by means of the Schuster closed circuit spirometer over 10 minute periods, the value for each period being represented by a horizontal section. It will be observed that immediately after the addition of synthalin the oxygen consumption of the preparation falls to one-half its previous value, and falls even lower towards the end of the experiment. Further, during this period of diminished oxygen consumption, which means also diminished combustion of sugar, the blood sugar level is actually falling. It is not merely, therefore, that we are unable to account for the fall in blood sugar by accelerated glycogen formation or combustion; oxidation is severely depressed and glycogen actually disappears. In estimating the lost glucose for which we have to account we must therefore add to the quantity represented by the fall in blood sugar level further quantities corresponding to the lost glycogen and to the fall in oxidation. We have to discover some means of disposal of all this glucose which does not involve absorption of oxygen and is not synthesis to glycogen.

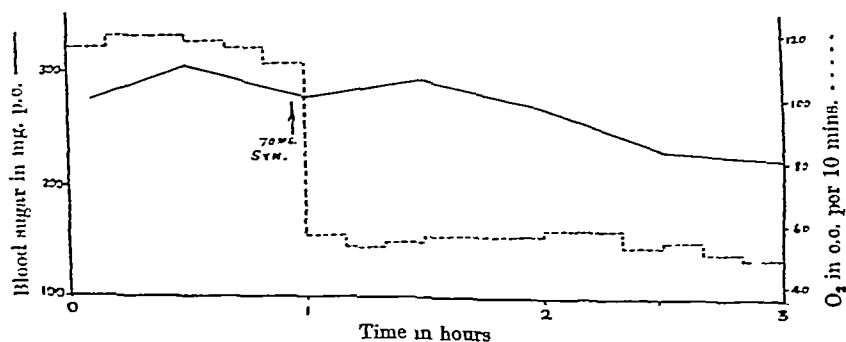


Fig. 5 (Exp. 21). Depression of metabolism in the eviscerated, spinal cat.

There is one reaction which suggests itself in explanation of the above facts, namely, the conversion of the sugar into lactic acid. An increase in lactic acid after synthalin has already been observed by Simola (10) in the blood of the whole animal. If this conversion does take place, we should expect the lactic acid formed to liberate an equivalent amount of carbon dioxide which would be given off in the expired air, so that we should obtain a respiratory quotient greater than unity. In Exp. 22, illustrated in Fig. 6, we measured the carbon dioxide production over three periods, in addition to the oxygen consumption, and the values

obtained, expressed in c.c. per 10 minutes, are shown in the diagram by the heavy horizontal lines A, B, and C.

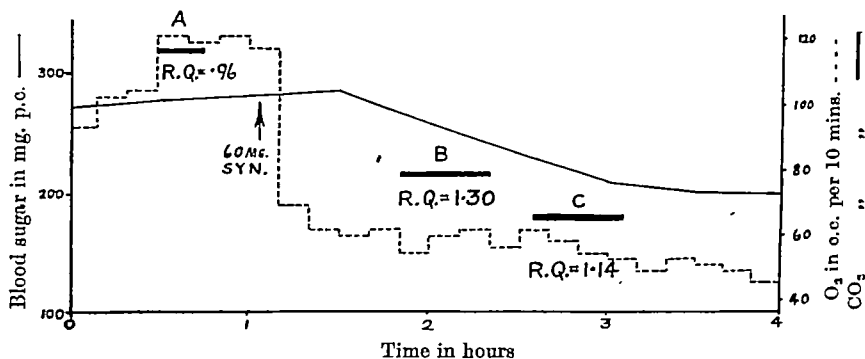


Fig. 6 (Exp. 22). Rise in the respiratory quotient.

It will be observed that, whereas before giving synthalin the volume of carbon dioxide produced was almost equal to that of the oxygen consumed, giving a respiratory quotient of 0.96, after giving synthalin it was produced in excess of the oxygen consumption, so that the respiratory quotient rose to 1.30 and 1.14. A similar rise in the R.Q., together with a fall in the oxygen consumption, has been observed clinically by Lublin (17).

Considering period B, there was produced, during 30 minutes, an excess of carbon dioxide amounting to 58 c.c. over that required to give an R.Q. of 1. If we assume that this carbon dioxide has been liberated as a result of the production of an equivalent quantity of lactic acid, it would indicate that 232 mg. of lactic acid have been formed. Now, during this period, the oxygen consumption is only half what it was before giving the synthalin, so that half the sugar which was previously being burnt has now to be accounted for in some other way. This amounts to 235 mg. In addition, there is the sugar disappearing from the blood, amounting to 150 mg., and the sugar corresponding to the glycogen disappearing from the muscles, of which we can make no estimate, so that we have to account for at least 385 mg. sugar. Although this considerably exceeds the calculated amount of lactic acid, we can hardly expect to arrive at a closer agreement without taking into consideration the change in the alkaline reserve of the blood and the tissues.

We have, however, performed three experiments in which the amount of lactic acid in the blood was followed. In these experiments the lactic acid content of the blood rose from 0.08 p.c. to 0.13 p.c., from 0.082 to

0.127 p.c. and from 0.110 to 0.175 p.c. during the first hour following the injection of synthalin, while in a control experiment without synthalin, the lactic acid content remained unchanged.

We cannot suppose that the lactic acid formed is confined to the circulating blood. It will be distributed through the total volume representing the blood and tissue fluids. A rough estimate made experimentally by Burn and Dale, in dealing with blood sugar, gave the figure of 500 c.c. per 3 kg. for this effective volume for the distribution of a diffusible substance in the body. This figure has already been used above in calculating the total glucose represented by a given fall in blood sugar percentage. Applying it again here, we obtained figures for the total lactic acid production of 250, 225 and 325 mg. of lactic acid in the experiments quoted.

Since the course of the metabolism and muscle glycogen were not followed in these experiments, it is impossible to say whether the total missing carbohydrate can be completely accounted for by the increase of lactic acid. The fact that later in the experiment the lactic acid content of the blood falls again, while the blood sugar is still declining, forbids the assumption that cleavage of glucose into lactic acid is the only change effected by synthalin, or the final stage in the metabolic process which it initiates. There is, however, sufficient evidence to support the conclusion that conversion of glucose to lactic acid is at least the most important of its immediate effects on preformed carbohydrate<sup>1</sup>.

### DISCUSSION.

In the present paper it is clearly demonstrated that synthalin given in a suitable dose, to the whole animal, lowers the blood sugar in a short time to a hypoglycæmic level; when this has been reached, death follows quickly, and relief of the hypoglycæmia appears only to postpone the fatal effect. The action seems to involve two factors. Firstly, an increased sugar disappearance, which is similar to that caused by insulin, in so far as it can be demonstrated in the perfused muscle preparation. Secondly, a direct action upon the liver, which manifests itself as a rapid depletion of the glycogen reserves, and a failure of the liver to react to adrenaline. In this respect the action of synthalin is quite different from that of

<sup>1</sup> Since sending the present paper to press we have received from Prof. Staub a report of an address(es) delivered by him in October, 1927, describing experiments very similar to those performed by us, and presenting similar conclusions. In addition, it is claimed that, in the eviscerated preparation, the whole of the sugar to be accounted for can be recovered as lactic acid in the muscles.



insulin. This difference extends to the effect on the muscles, for, whereas in the case of insulin, the disappearing sugar is deposited as glycogen in the muscles, synthalin causes a breakdown of muscle glycogen in addition to a disappearance of sugar.

The disappearing sugar is not burnt, since the respiratory metabolism actually falls under the action of synthalin, but the high respiratory quotients obtained suggest that it may have been converted into lactic acid. A production of lactic acid is actually observed, but it cannot be stated with certainty that this is sufficient to account for all the disappearing sugar. Possibly lactic acid is only an intermediate stage in the conversion of the sugar into some substance as yet unidentified.

The action on the liver, and perhaps that on the muscles, may be only one aspect of a general toxic action of synthalin which was also observed on the circulatory system as a fall of blood-pressure.

#### SUMMARY.

1. In the whole animal, synthalin causes a hypoglycæmia.
2. In the perfused muscle preparation, synthalin increases the disappearance of glucose.
3. The disappearing glucose is not converted into glycogen and is not burnt.
4. The respiratory quotient rises above unity.
5. The lactic acid content of the blood increases.
6. In the whole animal, the liver is depleted of glycogen, and is unable to react to adrenaline.
7. A toxic effect upon the liver and the circulatory system was observed.
8. Histological evidence of a toxic effect on the liver was obtained.

We wish to thank Dr H. H. Dale for his active help and interest in this investigation.

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# THE OXYGEN USAGE OF THE KIDNEY.

By A. R. FEE<sup>1</sup> AND A. HEMINGWAY.

(*From the Department of Physiology and Biochemistry,  
University College, London.*)

SEVERAL investigations of the metabolism of the kidney have been made to ascertain the mechanism and efficiency of that organ. Barcroft and Brodie<sup>(1)</sup>, Barcroft and Straub<sup>(2)</sup>, Tamura and Miwa<sup>(3)</sup> and others have measured renal oxygen consumption in the whole animal, and Bainbridge and Evans<sup>(4)</sup> made measurements upon the heart-lung-kidney preparation (the oxygen consumption of the heart and lungs, after separate determination, was deducted from that of the whole preparation). None of these methods, however, admitted of a continuous series of measurements over an extended period of time.

Recently the writers<sup>(5)</sup> confirmed the observation of Starling and Visscher<sup>(6)</sup> that the oxygen consumption of the heart-lung preparation bears, within reasonable limits, a direct linear relationship to the diastolic volume of the ventricles, and also showed that a similar relationship existed between the diastolic volume of the whole heart and the oxygen consumption of the preparation. An experiment was also given which demonstrated that this relationship was not destroyed if the heart-lung preparation were used to perfuse an isolated kidney. This led to the suggestion that if the (diastolic volume) : (oxygen consumption) ratio of a heart-lung preparation were determined before inserting a kidney into the circuit, subsequent measurements of diastolic volume and the total oxygen consumption of the preparation would afford data from which the oxygen usage of the perfused organ could be determined.

In this paper the experimental details of such a method of determining renal oxygen usage will be given along with the results which have already been obtained by its use.

## EXPERIMENTAL DETAILS.

### (A) *The closed-circuit. Heart-lung-kidney preparation.*

The heart-lung-kidney preparation as originally described by Bainbridge and Evans<sup>(4)</sup> and modified by Starling and Verney<sup>(7)</sup> was

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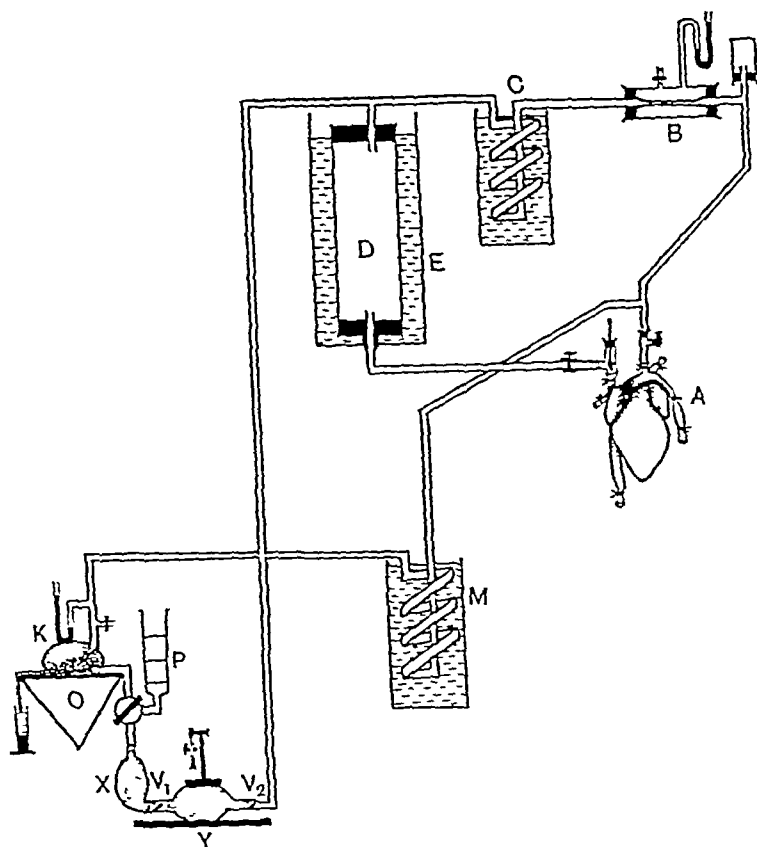


Fig. 1. Diagram of the closed circuit heart-lung-kidney apparatus.

*A, B.* Heart-lung preparation and resistance. *C, M.* Heating coils. *D.* Venous reservoir. *E.* Water bath. *K.* Kidney. *O.* Water-jacketed funnel. *P.* Measuring vessel. *X, Y.* Rubber bags of pump.  $\Gamma_1, \Gamma_2$ . Valves of pump.

Starling and Visscher<sup>(6)</sup>. The kidney (*K*) which is placed on a water-jacketed funnel (*O*) is connected to the arterial side of the heart-lung circuit by a cannula in the renal artery and to the venous reservoir (*D*) by a cannula in the renal vein.

In order to obtain the maximum advantage of the arterial pressure and to minimise the work of the heart in overcoming the resistance due to gravity, the kidney was placed as far as possible below the level of the heart. This necessitated the use of a pump to return the blood from

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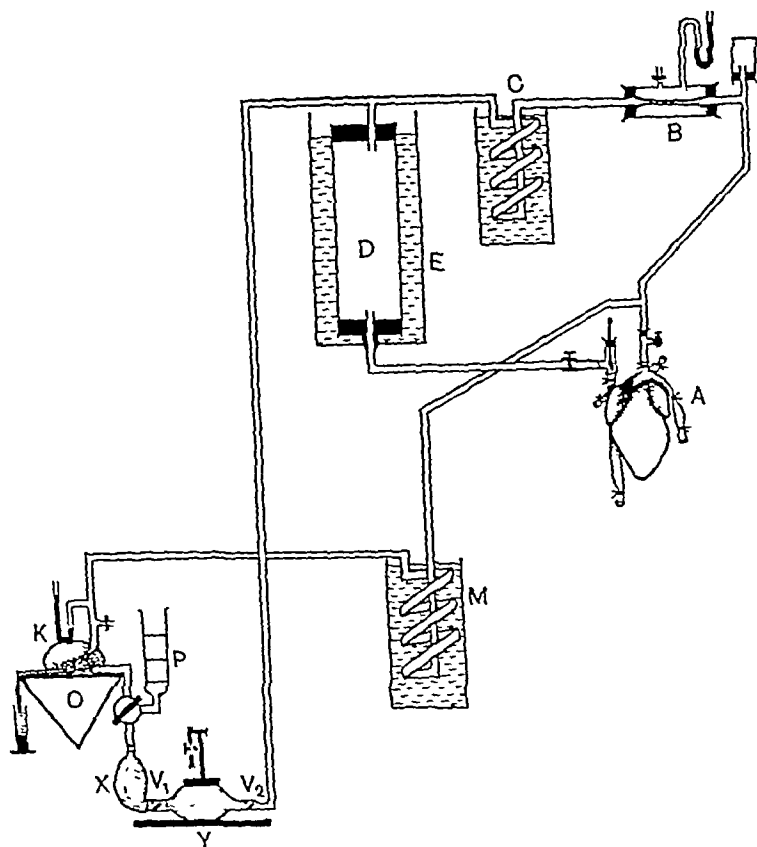


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In order to obtain the maximum advantage of the arterial pressure and to minimise the work of the heart in overcoming the resistance due to gravity, the kidney was placed as far as possible below the level of the heart. This necessitated the use of a pump to return the blood from

the kidney to the venous reservoir, a pump of such a type that would accommodate itself to variations in the renal blood flow. The device used consisted of two rubber bags having a capacity of 50 c.c. (Fig. 1, *X* and *Y*). As blood left the kidney it passed through the first bag (*X*) into the second (*Y*) from which it was expelled by a flat striker actuated by a crank shaft. The flap valves  $V_1$  and  $V_2$  directed the flow of blood. Any back pressure which might have developed on the renal side of the pump during the closure of  $V_1$  was not transmitted to the kidney owing to the distensibility of the walls of the first bag (*X*).

Renal blood flow was measured approximately by diverting the blood leaving the kidney into the graduated tube *P* by means of a three-way tap. As the tube *P* filled with blood there was a gradual, although slight, rise in the renal venous pressure; consequently this method of measurement did not give the unobstructed blood flow through the kidney, although changes in the rate were easily discernible.

The preparation of the closed heart-lung-kidney circuit was essentially the same as in the description given by Starling and Verney<sup>(7)</sup> with the exception that a cannula was placed in the renal vein as well as in the renal artery and ureter. The cardiometer used for measuring the diastolic volume of the heart was attached as described by Hemingway and Fee<sup>(5)</sup> after the heart-lung circuit had been completed. The only other modifications in technique necessary were those required to reduce the gradual leakage of blood which normally occurs in such a preparation. The œsophagus was tied off and several ligatures were placed around the thoracic aorta since this procedure was found to reduce the possibility of loss of blood through anastomotic channels. It was also necessary to ligature the perinephric tissue to avoid hæmorrhage after transferring the kidney to the heart-lung circuit.

It is worthy of note that the actual transference of the kidney to the heart-lung circuit could be effected in half a minute, the cannula being inserted into the renal vein immediately before the excision of the kidney. This was the only period during which the renal blood flow was arrested.

#### (B) *The measurement of oxygen consumption.*

Oxygen determinations were made by allowing a known volume of pure oxygen to enter a closed circuit in connection with the lungs and measuring the time taken for its consumption. Since carbon dioxide was continually removed with soda lime, any reduction in the volume of this closed circuit was an index of the amount of oxygen absorbed

by the blood in passing through the lungs. Such changes in extra-pulmonary gas volume were measured with a modified Krogh spirometer.

In Fig. 2, *B* is a water-jacketed burette capable of delivering accurately

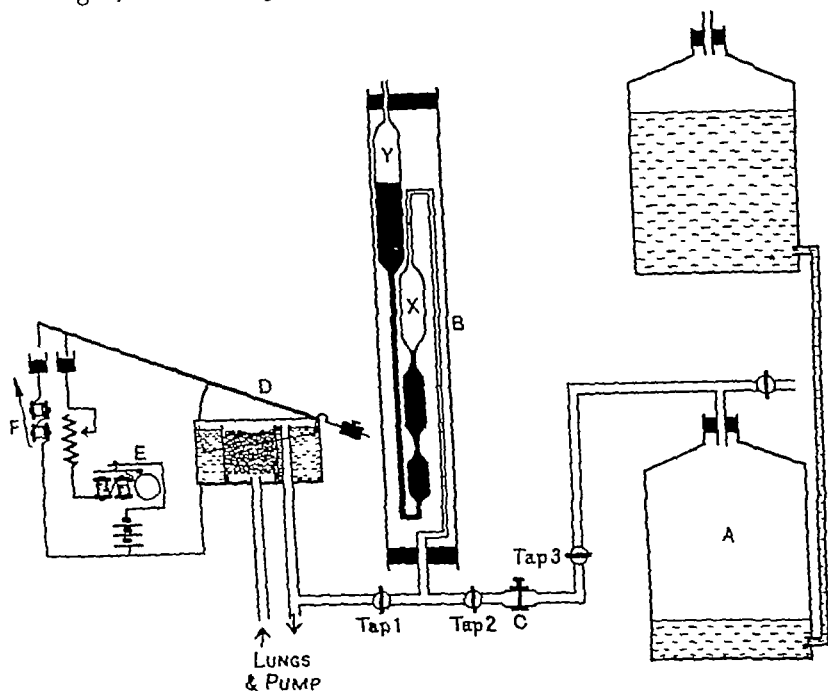


Fig. 2. Diagram of respiratory circuit.

*A.* Aspirator. *B.* Gas burette with bulbs *X* and *Y*. *C.* Fine adjustment for volume of gas in *B*. *D.* Spirometer. *E.* Bell. *F.* Signal marking on kymograph.

and quickly 20, 40, and 50 c.c. volumes of oxygen. By closing tap 1 and opening taps 2 and 3 oxygen will pass from the aspirator *A* into the burette expelling water from *X* into *Y*. When approximately the required volume has been forced in, tap 3 is closed and the water level in the burette allowed to settle. Before delivering the oxygen into the lung circuit this level is accurately adjusted by means of a clamp on a length of rubber tubing (*C*), and tap 2 is closed. Delivery is effected by opening tap 1. The absolute volume delivered will depend upon the temperature and barometric pressure as well as the difference in water level in the two arms of the burette. Corrections for these factors are necessary and also for the tension of aqueous vapour.

Air leaving the lungs passes through the respiration pump (the



“ideal” type as described by Starling(6) and enters the spirometer *D* where the carbon dioxide is removed by soda lime. As oxygen is removed by the blood circulating through the lungs the spirometer gradually falls and a lever bearing two contact points magnifies this change in level. When a certain point is reached the first contact closes a circuit which rings the bell *E*. This serves as a warning signal and shortly afterward the second contact is made and the signal *F* records on a kymograph. Since the resistance of this circuit is less than the first the bell also stops ringing. At this instant a stopwatch is started and the burette full of oxygen emptied into the circuit. While this oxygen is being used the burette is refilled in preparation for the following signal. The error of the gas delivery system is less than 1 p.c.

The main objection to measurements of oxygen usage by this system is that any change in lung volume is reflected in the movements of the spirometer. Extreme care must be taken in preparing the heart-lung circuit as rough handling of the lungs causes them to leak appreciably. It is advisable to avoid increasing the stroke of the respiration pump beyond the absolute minimum until all operative manipulations have been completed. The slightest œdema of the lungs vitiates the readings, but is easily detected by the marked disagreement of consecutive readings taken under the same conditions. The development of any leak in the gas circuit during the perfusion of the kidney is readily detected by determining the (diastolic volume) : (oxygen consumption) ratio of the heart-lung preparation alone at the end of the experiment and comparing it with that determined at the beginning.

## EXPERIMENTAL RESULTS.

### (A) Chloride excretion.

Starling and Verney(7) have shown that the isolated kidney perfused by the heart-lung preparation gradually approaches a condition similar to that found in *diabetes insipidus*, inasmuch as large amounts of a markedly hypotonic urine are excreted. Whereas the urinary chlorides in the normal intact animal are usually above 0.6 gm. p.c. as NaCl, at the end of an hour's perfusion the isolated kidney often produces a urine containing as little as 0.03 gm. p.c. The absolute output of chloride usually falls also, but this is not always so owing to the diuresis which occurs.

The renal oxygen consumption, as measured by the method just described, increases as this condition is attained. The results of a typical

experiment are given in Table I. There is a fall both in the percentage and absolute amounts of chloride excreted per unit time, an increase in the rate of urine flow and also in the renal oxygen consumption.

TABLE I.

Time	Blood flow c.c./min.	O <sub>2</sub> usage c.c./min.	Time	Urine c.c./10 min.	Urinary chloride as NaCl gm. p.c.
1.30-1.40	88	0.70	—	—	—
1.40-1.50	—	1.14	1.40-1.52	1.6	0.05
1.50-2.00	125	1.13	1.52-2.03	7.2	0.01
2.00-2.10	135	2.04	2.03-2.13	10.0	trace

Weight of kidney 37 gm.

The average oxygen consumption in the above experiment is 0.034 c.c. per gm. per min. with a minimum consumption of 0.019 c.c. and a maximum of 0.055 c.c. In most experiments performed the oxygen consumption fell between a minimum oxygen consumption of 0.03 c.c. and a maximum of 0.20 c.c. per gm. per min. Bainbridge and Evans(4) found an average renal oxygen consumption of 0.045 c.c., while Barcroft and Straub(2) obtained values between 0.03 c.c. and 0.10 c.c. per gm. per min.

(B) *The effect of changes in arterial pressure.*

Barcroft and Straub (*loc. cit.*) in their investigation of changes in oxygen consumption during different types of diuresis made no inquiry into the relationship between the arterial pressure and the gaseous metabolism of the kidney, only noting that an increase in arterial pressure resulted in an increased urine flow. Starling and Verney(7) also showed the dependence of urine flow upon the arterial pressure, and Richards and Plant(9) demonstrated that the rate of blood flow was of little importance in the rate of formation of urine when compared to the arterial pressure.

By altering the resistance of the heart-lung circuit we were able to alter the pressure at which the kidney was perfused. Any alterations in diastolic volume of the heart which occurred as a result were minimised by suitable adjustments of the venous inflow in order to obviate any error which might be introduced in the calculation of the cardiac oxygen consumption from the (diastolic volume) : (oxygen consumption) ratio. The results of such an experiment are given in Table II. It can be seen that there is a distinct parallelism between the blood pressure and oxygen usage. It must also be remembered that the gradual increase in renal oxygen consumption during the perfusion of an isolated kidney

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TABLE II.

Oxygen consumption			Urine analysis		
Time	O <sub>2</sub> usage c.c./min.	Arterial pressure mm./Hg	Time	Urine c.c./10 min.	Chloride as NaCl gram. p.c.
1.38-1.43	1.13	84	—	—	—
1.43-1.49	1.14	82	—	—	—
1.49-2.04	3.46	112	—	—	—
2.04-2.08	—	114	—	—	—
2.08-2.12	3.63	113	—	—	—
2.12-2.15	3.63	112	2.11-2.21	3.4	0.10
2.15-2.19	3.53	108	—	—	—
2.19-2.23	3.36	105	2.21-2.30	3.3	0.04
2.23-2.27	3.36	—	—	—	—
2.27-2.31	3.86	110	—	—	—
2.31-2.34	4.02	110	2.30-2.40	4.1	0.03
2.34-2.41	4.43	126	—	—	—
2.41-2.44	4.70	126	2.40-2.52	5.3	0.03
2.44-2.48	4.90	126	—	—	—
2.48-2.54	3.61	94	2.52-3.02	1.6	0.05
2.54-2.58	3.54	94	—	—	—
2.58-3.02	3.54	94	—	—	—
3.02-3.06	—	94	3.02-3.07	1.0	0.05
3.06-3.10	3.03	94	3.07-3.12	0.7	0.02

First urine sample rejected to allow for "dead space" in renal pelvis and cannula.  
Weight of kidney approx. 25 gm.

which has just been described is also taking place, consequently the rate of oxygen consumption towards the end of the experiment will be relatively higher than at the beginning.

### (C) *The effect of pituitary extract.*

Before the effect of pituitary extract upon the kidney could be measured it was necessary to determine if the addition of the extract to the circulating blood would in any way alter the (diastolic volume) : (oxygen consumption) ratio of the heart. Four experiments were carried out upon the heart-lung preparation to control this possible source of error, and in none of them could any appreciable change in the ratio be detected except immediately after the injection of the pituitrin. One of the experiments is given in Table III. Moreover in an experiment

TABLE III.

Time	Diastolic volume of heart	Oxygen usage c.c./min.
1.15-1.30	$\alpha$ plus 12.7 c.c.	12.67
1.30-1.39	" ?	14.23
1.39-1.55	" 12.5 c.c.	12.64

Heart weight 80 gm. Approx. 800 c.c. blood in circulation. Blood flow 400 c.c. per min. A quarter unit of B.D.H. pituitrin added at 1.30.

given in a previous paper (5) the oxygen usage of a heart-lung preparation was redetermined after it had been used to perfuse an isolated kidney for two and a half hours. During this period three quarter-unit amounts of pituitary extract had been added to the circulating blood. The observed cardiac oxygen consumption was 8.10 c.c. per min. and the value calculated from the preliminary data 7.92 c.c. per min.

The effect of pituitary extract upon the renal oxygen consumption is illustrated in Table IV. A quarter unit of B.D.H. extract was added

TABLE IV.

Oxygen consumption			Urine analysis		
Time	O <sub>2</sub> usage c.c./min.	Blood flow c.c./min.	Time	Urine c.c./10 min.	Chloride as NaCl gram. p.c.
1.10-1.20	4.14	68	1.10-1.20	1.9	0.05
1.20-1.30	4.36	79	1.20-1.30	3.3	0.07
1.30-1.40	4.78	79	1.30-1.40	3.1	0.06
1.40-1.50	5.06	—	1.40-1.50	3.1	0.05
1.50-1.55	5.30	75	1.50-2.05	2.5	0.15
1.55-2.05	4.76	71	—	—	—
2.05-2.10	—	68	2.05-2.15	1.8	0.22
2.10-2.15	2.92	—	—	—	—
2.15-2.25	3.72	—	2.15-2.25	1.8	0.17

Weight of kidney 19 gm. Approx. 1 litre of blood in circulation. A quarter unit of B.D.H. pituitrin added at 1.53 and 2.08. Blood-pressure throughout experiment 122 mm. Hg.

at the times indicated. Approximately a litre of blood was in circulation. There was an immediate increase in the percentage and absolute excretion of chlorides and a diminution in the urine flow and renal oxygen consumption. The arterial pressure was constant throughout the experiment and practically no change in blood flow occurred after the addition of such a small amount of the extract.

#### SUMMARY.

1. A method of measuring the oxygen consumption of the perfused isolated kidney is described.
2. The oxygen consumption of such a kidney varies between 0.03 and 0.20 c.c. per gram. of kidney tissue per min.
3. Associated with the gradual change in the volume of and chloride concentration in the urine produced by the isolated perfused kidney there is a gradual rise in the renal oxygen consumption.
4. An increase in arterial pressure causes a rise in the renal oxygen consumption.
5. Following the addition of small amounts of pituitary extract to the blood circulating through an isolated kidney the changes in the



chloride concentration and volume of urine are associated with a fall in the renal oxygen consumption.

This investigation was begun under the supervision of the late Professor E. H. Starling who was largely responsible for the successful development of the experimental technique employed.

The expenses of the research were defrayed by a grant from the Royal Society.

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## THE ENZYMIC PROCESSES IN MAMMALIAN SKELETAL MUSCLE.

BY MYRA K. BEATTIE, J. BELL AND T. H. MILROY.

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WITHIN recent years our knowledge of the ferment changes in muscle has been greatly advanced by the investigations of Meyerhof and his co-workers(1). The characteristic transformations which glycogen undergoes in intact muscle have been shown to occur not only in minced muscle but also in certain extracts of muscle which have been so prepared as to remove practically all the protein. Such solutions, containing the active ferments, can be employed for the study of the chemical and physico-chemical changes associated with the degradation of glycogen under conditions which enable a much simpler analysis to be made than in the case of more heterogeneous mixtures, such as minced muscle in saline solutions of different composition. The investigations which dealt with minced muscle or the expressed juice of muscle have however not only constituted the starting point for the later investigations, but are themselves of very great value in enabling a comparison to be made between the enzymic changes in colloidal and non-colloidal mixtures. The work of Embden and the Frankfurt school directed attention for the first time to the significance of phosphoric acid esters of the sugar as precursors of lactic acid.

From the investigations of the expressed juice, which was the early subject of study by Embden, Kalberlah and Engel(2), it became evident that lactic acid was more readily produced from those phosphoric acid esters than from glycogen, so that it appeared as if the process of esterification were normally a function of the muscle tissue elements. Embden and Haymann(3), however, showed that the esterification process could go on in the expressed juice if fluoride were added, and that on the addition of glycogen to the juice in the presence of this anion the formation of a stable ester from the free phosphate was as readily brought about as in minced muscle. When glucose or maltose were added under those conditions there was no esterification. That

added glycogen could be broken down to lactic acid by the juice in the absence of fluoride was therefore probable if only the conditions were rendered satisfactory. As Kondo (4) pointed out, the increase in acidity in the expressed juice checked glycolysis, and therefore he added sodium bicarbonate to increase the buffering capacity. Until, however, the reaction changes were accurately determined it was difficult to judge as to whether the buffering were sufficient and also the phosphate supply adequate to enable the ferment changes to proceed. Therefore, before considering the changes which occur when saline extracts of cooled minced muscle are used the action of the diluted expressed juice will be dealt with.

*Enzymic properties of the juice.*

In some cases the juice was expressed from muscle dropped into liquid air, but, as too long a time was spent in subsequently obtaining the juice, it was found to be more convenient to proceed in the following way. After the rabbit was bled the limbs were embedded in an ice-salt mixture. When the underlying tissue had cooled, the muscles were exposed carefully, removed with as little injury as possible and placed in a dish surrounded by a mixture of powdered ice and ammonium nitrate. The muscle was then minced, mixed with purified silver sand and as quickly as possible subjected to pressure in a Buchner filter press. The juice was collected in glass cylinders, immersed in the ice-salt mixture, quickly centrifugalised and the supernatant fluid used for investigation. Three mixtures containing the juice were prepared, the first (*A*) mixed with twice its volume of 0.15 *N* KCl, the second (*B*) with twice its volume of 0.3 molar  $\text{Na}_2\text{HPO}_4$ , and the third (*C*) with the same phosphate along with an addition of 6 mg. glycogen per c.c. juice. A small quantity of thymol was added to the incubating mixtures.

The reaction and composition of *A* (one part juice plus two parts 0.15 *N* KCl) before incubation were as follows:

1. Reaction pH 6.35.
2. Protein 2 p.c.
3. Creatine 0.014 molar.
4.  $\text{H}_3\text{PO}_4$  (a) In labile form (phosphagen) 0.007 molar.  
     (b) Total free phosphate 0.0124 molar (Embden) (same value Fiske-Subarrow method).  
     (c) Preformed hexose phosphate 0.0061 molar ( $\text{H}_3\text{PO}_4$ ).
5. Lactic acid 0.0235 molar.

*Electrometric and analytical methods.*

1. All reaction determinations were made with the hydrogen electrode and with a 3.5 *N* KCl calomel electrode as the reference one.
2. Protein. From difference between total nitrogen and non-protein nitrogen after deproteinisation with trichloroacetic acid.
3. Creatine. By Folin's method after deproteinisation by colloidal iron and transformation into creatinine.
4. (a) Labile phosphate. "phosphagen" of the Eggletons(6). The difference between the zero time value by the Fiske-Subarrow method (F. S.)(5) and the final value.
- (b) Total free phosphate by Embden's gravimetric method (E.) and from the final F. S. value.
- (c) Preformed hexose phosphate. Difference between the initial value obtained by the E. method and the free phosphate value after warming in bicarbonate solution at 40° for two hours.

*Incubation of this mixture at 30° for 2 hours.*

Before incubation the reaction was brought to pH 7.20 by addition of  $M/5$  NaOH, the necessary corrections being made in the subsequent analyses for the slight alteration in volume.

The changes produced during incubation:

1. Reaction shifted from pH 7.20 to pH 6.92.
  - 2 a. The labile phosphate (phosphagen) fell from 0.007 to 0.0048 molar.
  - 2 b. The total free phosphate increased from 0.0124 to 0.0185 (E.) (0.0180 F. S.) molar.
  - 2 c. The pre-existing hexose phosphate had all broken down.
  3. Increment in lactic acid from 0.0235 to 0.0265 molar.
- Creatine value not redetermined.

In order to arrive at a knowledge of possible changes in buffering capacity during incubation, the original mixture, the same mixture after incubation and a pure phosphate solution of the same molar concentration as the total free phosphate in the incubated mixture were electrometrically titrated by known base or acid additions, the alterations in pH being observed so that the base molar increment per unit pH shift within the zone pH 6.35 to 7.24 might be given as the ratio of the observed differences  $\Delta B/\Delta pH$ .

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Creatine value not redetermined.

In order to arrive at a knowledge of possible changes in buffering capacity during incubation, the original mixture, the same mixture after incubation and a pure phosphate solution of the same molar concentration as the total free phosphate in the incubated mixture were electrometrically titrated by known base or acid additions, the alterations in pH being observed so that the base molar increment per unit pH shift within the zone pH 6.35 to 7.24 might be given as the ratio of the observed differences  $\Delta B/\Delta pH$ .

TABLE I. Electrometric titration. (Fig. 1.)

I = A before incubation.				II = A' after incubation.			
B (base addition M. per litre)	pH	$\Delta B/\Delta pH$	Mean pH	B (base addition M. per litre)	pH	$\Delta B/\Delta pH$	Mean pH
—	6.35	—	—	—	6.35	—	—
0.005	6.56	0.0238	6.45	0.004	6.63	0.0142	6.49
0.010	6.82	0.0192	6.69	0.009	6.93	0.0166	6.78
0.015	7.04	0.0227	6.93	0.014	7.24	0.0161	7.08
0.020	7.37	0.0151	7.20				

III = 0.0185 molar phosphate in 0.15 N KCl.

B (base addition M. per litre)	pH	$\Delta B/\Delta pH$	Mean pH
—	6.35	—	—
0.005	6.83	0.0104	6.59
0.0075	7.08	0.0100	6.95
0.0095	7.33	0.0080	7.20

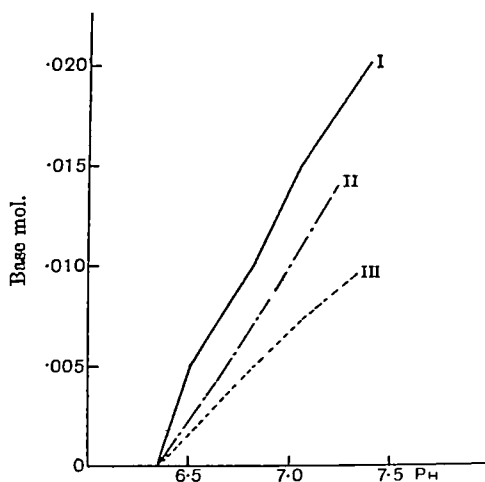


Fig. 1.

I = A before incubation.      II = A' after incubation.      III = 0.0185 molar phosphate.

The mean buffering values (van Slyke(7)) of the three systems within the reaction zone from pH 6.35 to 7.24 are (1) 0.0202; (2) 0.0157; (3) 0.0092.

The greater buffer values of the mixtures are due to their protein content, and the reason for the fall on incubation is the partial separation of protein during the incubation period and the removal of the same by filtration prior to the titration.

*B. The mixture of juice and phosphate.*

One part of the juice with a total free phosphate (labile and stable) value of 0.0372 molar was mixed with two parts of 0.0296 molar  $\text{Na}_2\text{HPO}_4$ . On analysis by E. method, the phosphate concentration of the mixture was found to be 0.0326, and on warming in bicarbonate solution it rose to 0.0387 molar.

Composition of *B* mixture before incubation:

Protein and creatine values same as in *A* mixture.

Free phosphate (total) 0.0326 molar.

Labile phosphate (phosphagen) 0.007 molar.

Hexose phosphate 0.0061 molar.

Reaction pH 6.62. Before incubation the reaction was brought to pH 7.20.

After incubation at 30° for 2 hours the changes were:

1. Rise in total free phosphate from 0.0326 to 0.0387 molar.
2. Breaking down of all the pre-existing hexose phosphate.
3. Rise in lactic acid from 0.0214 to 0.0278, that is to say practically equimolecular increments of phosphoric and lactic acids.
4. Diminution in alkalinity from pH 7.20 to pH 6.94.

The mixtures before and after incubation, as well as a pure phosphate solution of the same total concentration (0.0387 M.), as was present in the incubated mixture, were electrometrically titrated.

TABLE II. (Fig. 2.)

I = <i>B</i> before incubation.				II = <i>B'</i> after incubation.			
<i>B</i>	pH	$\Delta B/\Delta pH$	Mean pH	<i>B</i>	pH	$\Delta B/\Delta pH$	Mean pH
—	6.06	—	—	—	5.98	—	—
0.0064	6.35	0.022	6.20	0.0125	6.54	0.0223	6.26
0.0125	6.62	0.022	6.48	0.025	6.94	0.0312	6.74
0.0250	6.95	0.0378	6.78				
IV = 0.0387 molar phosphate.							
<i>B</i>	pH	$\Delta B/\Delta pH$	Mean pH				
—	6.00	—	—				
0.0046	6.35	0.0131	6.17				
0.0093	6.60	0.0185	6.47				
0.0170	6.95	0.0220	6.77				

It is evident that the changes which have occurred on incubation of this mixture, containing additional phosphate but no added glycogen, are simply those concerned in the breaking down of the pre-existing hexose phosphate. The slight acid change is due to the lactic acid formation, and the electrometric titrations show that the mixtures behave as inorganic phosphate systems with an added buffer value due mainly to the colloid present. Both before and after incubation the maximal



buffering zone is slightly on the acid side of neutrality, about  $pH$  6.8, that is to say in the region of the second dissociation constant of  $H_3PO_4$ .

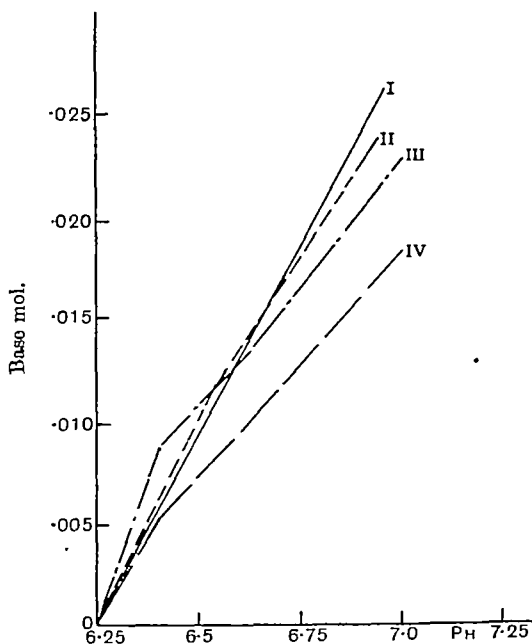


Fig. 2.

I =  $B$  (juice phosphate) before incubation. II =  $B'$  (juice phosphate) after incubation.  
 III =  $C'$  (juice phosphate and glycogen) after incubation. IV = 0.0387 M. phosphate.

### C. Mixture of juice and phosphate with added glycogen.

The mixture was of the same composition as  $B$ , only it contained in addition 6 mg. glycogen per c.c. mixture. The composition was the same as that of  $B$  before incubation. The reaction was  $pH$  6.62, and this was brought to  $pH$  7.2 before placing in the thermostat at  $30^\circ$ . After 2 hours' incubation the changes were as follows:

$C'$ . (1) Fall in free phosphate from 0.0326 to 0.030 molar (E.). By the F. S. method, the final value was much lower, namely, 0.022 molar. As the total phosphate (free and bound) was originally 0.0387, this would give by the E. method 0.030 free and 0.0087 bound, and by the F. S. method 0.0220 free and 0.0167 bound. Such a marked disparity in the values obtained by the two methods was observed in no other case<sup>1</sup>.

<sup>1</sup> Probably the "Emden" value is the correct one. If so, some experimental error was made in the F.S. determination as in other analyses the results obtained by the two methods were practically identical.

Phosphate bound in labile form was very small—0.002 molar.

(2) A great increase in lactic acid production, namely, from 0.0214 to 0.045 molar.

(3) Marked increase in acidity from pH 7.20 to 5.93.

TABLE III. Electrometric titration. *C'* (Fig. 2 III).

B	pH	$\Delta B/\Delta pH$	Mean pH
—	5.93	—	—
0.010	6.40	0.0212	6.16
0.015	6.62	0.0227	6.51
0.025	6.99	0.0270	6.80

The buffer values of this incubated mixture differ from those of *B'* in the range from 6.62 to 6.99 pH. The lower value of the buffering capacity of *C'* in this zone is probably mainly due to the partial replacement of the weaker acid, which would exercise its main buffering action in this zone, by acid or acids with a slightly higher dissociation constant.

The mean buffering values of the systems considered between pH 6.35 and 7.24 are as follows:

*B* (and *C*) before incubation 0.0332,

*B'* after incubation 0.0294,

*C'* after incubation 0.0255,

Phosphate solution 0.0387 M, 0.0204.

The higher buffer values (B.V.) of the mixtures are due to the protein present.

It is evident that the juice diluted with phosphate can increase glycolysis when glycogen is added. In the absence of added glycogen, lactic acid production follows the break-down of the pre-existing hexose phosphate and is proportional to the free phosphate increment.

The muscle tissue left after the expression of the juice was also found to possess enzymic properties, and the action of a saline extract of this mass will be briefly referred to. The extract was made by rapid extraction of the muscle (immediately after expression of the juice) with a 0.15 *N* KCl at  $-4^{\circ}$ . This was diluted with an equal volume of 0.05 molar,  $\text{Na}_2\text{HPO}_4$  containing 12 mg. glycogen per c.c. extract. The reaction of the mixture was pH 7.47.

Composition	Before incubation	After 2 hours at $30^{\circ}$
pH	7.47	6.70
Total free phosphate (E. and F. S.)	0.0263 M.	0.0163 M.
Hexose phosphate	(mere trace)	0.010 "
Lactic acid	0.0046 "	0.0113 "
Protein	0.38 %	0.38 %

*Electrometric titration.*

As the mixture before incubation behaved almost exactly like a 0.0263 m. phosphate, the B.V. of such a solution will be compared with those of the mixture after incubation.

TABLE IV.

0.0263 m. phosphate.				The mixture after incubation.			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
—	6.02	—	—	—	6.02	—	—
0.00275	6.33	0.0090	6.17	0.0045	6.33	0.0145	6.17
0.00775	6.70	0.0135	6.51	0.0095	6.70	0.0135	6.51
0.00375	7.09	0.0152	6.89	0.0145	7.09	0.0128	6.89

In the range pH 6.33 to 6.70 the B.V. was the same in the two, on the acid side of this range it fell in the phosphate and rose in the mixture, while on the alkaline side the reverse change occurred. As the protein content of the mixture was very low, the B.V. is practically entirely due to the phosphate present and is evidently of the same order in both, only, owing to the partial replacement of the free phosphate by the phosphoric acid esters with slightly higher dissociation constants, the zone of maximal buffering has shifted from the alkaline towards the acid side.

When glycogen is added therefore to saline extracts of the muscle tissue from which the juice has been expressed, the characteristic processes accompanying glycolysis, viz. esterification, lactic acid production, alterations in B.V. are all to be observed.

*Enzymic activity of saline extracts of muscle.*

Suitable methods for the preparation of extracts which possess active glycolytic properties have been described by Meyerhof. The factors influencing enzymic activity have been carefully investigated and the results described in a series of valuable papers. Among the most important discoveries was the detection of the rise in acidity which accompanies the process of esterification. In order to obtain accurate knowledge regarding the phosphoric acid esters of the hexoses, he determined the dissociation constants of a number of these acids and made use of the information so gained for the elucidation of the reaction changes accompanying enzymic glycolysis. For a period of rather more than a year we have been engaged in the study of similar extracts, especially of mammalian skeletal muscle, and the results, based on a very large number of experiments, were found to be in the main confirmatory of those obtained by Meyerhof. Attention was directed largely to the

significance of the reaction shifts in the incubated muscle extracts, but in order to understand the nature of these alterations it was necessary to follow the chemical changes which accompany the reaction displacements. Before referring to the changes in reaction, it is advisable therefore to summarise briefly the chemical transformations which occur in the extracts alone when incubated, and the alterations produced when various carbohydrates are added to these with and without an addition of free phosphate.

The reaction changes in selected examples will then be dealt with and their significance discussed. The extracts were made in a variety of ways. In practically all cases pale muscles of the rabbit were taken, frozen, minced and extracted either by the methods employed by Meyerhof or by slight modifications of them. For example, in some cases the minced muscle was mixed with powdered ice or in certain cases with saline solutions containing glycogen or starch. The proportion of minced muscle to extracting fluid varied, usually two parts of muscle to three parts of extracting fluid. The average composition of these extracts may be stated as follows:

Protein nitrogen 0.1 p.c.

Creatine 0.007 molar.

Total free phosphate about the same as the creatine value.

Hexose phosphate 0.001 to 0.003 molar ( $H_3PO_4$ ).

Labile phosphate (phosphagen) about 0.002 molar.

Reducing values (R.V.) (Hagedorn-Jensen) in terms of glucose 0.30 to 0.98 mg. per c.c.

The extracts, however, varied greatly in their composition depending upon the period used for extraction, as will be seen from the following table, giving in terms of mg. per c.c. the amounts of free  $H_3PO_4$  (by the E. method), lactic acid and reduction value in terms of glucose (by Hagedorn-Jensen).

TABLE V.

	Mg. per c.c.		Average
	Maximum	Minimum	
$H_3PO_4$ (free)	1.02	0.43	0.753
Lactic acid	1.42	0.42	0.830
R.V.	0.98	0.30	0.470

*Chemical changes in the extracts.*1. *Without addition of phosphate or glycogen.*

In order to allow the ferment changes to proceed the extracts were rendered alkaline before incubation by the addition of  $\text{NaHCO}_3$  up to 0.025 molar concentration.

The results of incubation at  $22^\circ$  for periods varying from 1 to 4 hours are given in the following table:

Original values. Mg. per c.c.			Incubation period. Hours	Changes after incubation. Mg. per c.c.		
$\text{H}_3\text{PO}_4$	Lactic acid	R.V.		$\text{H}_3\text{PO}_4$	Lactic acid	R.V.
*1. 0.65	0.81	0.41	1	+0.22	+0.29	+0.11
2. 0.84	0.69	0.40	1	+0.18	+0.13	+0.22
*3. 0.65	0.81	0.41	2	+0.30	+0.28	-0.06
4. 0.67	0.85	0.67	3	+0.12	+0.20	-0.11
5. 0.68	0.94	0.56	4	+0.28	+0.20	-0.21

\* The same extract incubated for different periods.

In all cases there was found to be an increase in free phosphate (+) accompanied by a rise in lactic acid (+), the increments being approximately equimolecular, indicating therefore the breakdown of a hexose diphosphate. A diminution in the R.V. of the extract was the general rule, although in a few instances where the period of digestion was short the reverse occurred. The latter may be accounted for by hydrolysis of the glycogen originally present.

2. *After addition of glycogen to the extracts quite different results were obtained, as shown in the following table:*

	Original values. Mg. per c.c.			Glycogen added. Mg. per c.c.	Incubation period (at $22^\circ$ ). Hours
	$\text{H}_3\text{PO}_4$	Lactic acid	R.V.		
1.	0.78	1.42	—	6	2
2.	0.65	0.81	0.41	6	2
3.	0.67	0.85	0.67	6	3
4.	0.68	0.94	0.56	12	4
*5.	0.67	0.81	0.66	—	4

	Changes after incubation. Mg. per c.c.			Glycolysis Esterifica- tion
	$\text{H}_3\text{PO}_4$	Lactic acid	R.V.	
1.	-0.69	—	—	—
2.	-0.28	+0.37	+0.57	1.47
3.	-0.25	+0.28	+1.25	1.20
4.	-0.68	+0.58	+2.84	0.93
*5.	-0.67	+0.51	+2.64	0.82

\* In this case a 0.15 N KCl solution containing 1 p.c. glycogen was used for extraction.

The ratio of glycolysis to esterification stated in terms of hexose is given in the last column.

After a period of digestion, lasting from two to four hours, there was a decrease in free phosphate (—), in some cases amounting to its complete disappearance. The diminution in phosphate was always accompanied by an increment in lactic acid greater than occurred in the extract alone, and roughly equimolecular with the phosphate which had disappeared, so that the ratio of glycolysis to esterification was approximately 1. Hydrolysis of the added glycogen naturally caused a decided rise in the reduction value.

It was evident, therefore, from these results that the failure of the original extract to form the esters was due to lack of glycogen, as addition of glycogen allowed a marked synthesis to occur, and when, through lack of the hexose precursor, the ester was not formed, instead of synthesis there was a breakdown of the pre-existing phosphoric acid ester. The fact that when glycogen was added to the extract the amount of hexose changed into lactic acid was roughly equal to the amount esterified indicates the formation primarily of a labile ester, a hexose monophosphate, half of the hexose component being transformed into lactic acid, and the other half stabilised as a hexose diphosphate. It was noticed that in none of our experiments did the lactic acid increment after digestion rise above the original phosphate value even when an excess of glycogen was added, which confirms the supposition that lactic acid is never formed directly from glycogen, but must be derived from an intermediate phosphoric acid ester.

3. *The changes produced when both precursors of the ester were added are shown in the following table:*

TABLE VIII.

	Original values. Mg. per c.c.			Additions. Mg. per c.c.		Incubation period (at 20° to 25°). Hours
	H <sub>2</sub> PO <sub>4</sub>	Lactic acid	R.V.	Glycogen	H <sub>2</sub> PO <sub>4</sub>	
1.	2.95	0.81	0.41	6	2.26	2
2.	3.04	1.42	—	6	2.26	2
3.	2.94	0.85	0.67	6	2.26	3
4.	6.22	0.99	0.46	6	5.3	4
4 a.	6.22	0.99	0.46	12	5.3	4
4 b.	11.52	0.99	0.46	12	10.6	4
5.	5.60	0.65	0.43	12	5.3	3
6.	5.88	0.94	0.56	12	5.3	4
7.	3.19	1.05	0.45	3	2.65	3
8.	5.97	0.81	0.66	6	5.3	4
9.	6.21	1.14	0.82	3	5.3	4
9 a.	6.21	1.14	0.82	6	5.3	4
9 b.	11.51	1.14	0.82	6	10.6	4

TABLE VIII (contd.).  
Changes after incubation.  
Mg. per c.c.

	$H_3PO_4$	Lactic acid	R.V.	Glycolysis Esterifica- tion
1.	-0.70	+0.86	+0.77	0.55
2.	-1.87	+0.87	—	0.52
3.	-1.42	+0.21	+0.92	0.16
4.	-2.52	+0.49	+0.94	0.21
4 a.	-5.10	+3.38	+1.77	0.76
4 b.	-6.91	+2.80	+1.71	0.45
5.	-1.50	+0.59	+2.39	0.45
6.	-4.31	+1.50	+2.64	0.38
7.	-3.11	+1.05	+1.64	0.36
8.	-5.39	+0.71	+2.46	0.14
9.	-2.91	+1.49	+2.00	0.55
9 a.	-5.81	+5.30	+2.74	1.0
9 b.	-8.42	+3.33	+2.38	0.43

Nos. 7 to 9 b are glycogen extracts.

When phosphate and glycogen were added to these extracts both esterification and glycolysis were increased, but esterification was now dominant. The average ratio of glycolysis to esterification was now 0.43. The best esterification and glycolysis were obtained when an excess of glycogen was present along with the added phosphate. In Nos. 4, 4 a and 4 b, which refer to the same extract, doubling the quantity of glycogen resulted in almost complete disappearance of the free phosphate and a large increment in lactic acid, while by doubling the phosphate as well as the glycogen (4 b) there is a further esterification, but lactic acid formation is checked earlier. The same is seen in the glycogen extracts (Nos. 9, 9 a and 9 b).

The glycogen KCl extracts, when compared with the KCl extracts to which glycogen is added later, appear to be more active as regards both esterification and glycolysis, but the ratio of glycolysis to esterification is about the same. The greatest ester formation was reached in the glycogen extract, 8.4 mg.  $H_3PO_4$  per c.c. extract disappearing, while in the KCl extract the maximal amount bound was 6.9 mg.  $H_3PO_4$  per c.c. The highest lactic acid formation in the glycogen extract was 5.3 mg. per c.c., in the KCl extract 3.38 mg. per c.c.

4. *The effects of addition of sodium fluoride to the extracts are shown in Table IX.*

In the mixtures containing fluoride there was no evidence of lactic acid production, the slight differences between the pre- and post-incubation values lying within the region of experimental error.

A constant feature in the presence of fluoride was the increased

TABLE IX.

1-4 in the absence of fluoride at 22°.

1 a-4 a in the presence of fluoride at 22°.

	Before incubation.			Additions.		
	Mg. per c.c.			Mg. per c.c.		
	H <sub>3</sub> PO <sub>4</sub>	Lactic acid	R.V.	H <sub>3</sub> PO <sub>4</sub>	Glycogen	NaF %
1.	5.60	0.65	0.43	5.17	12	—
1 a.	5.60	—	0.43	5.17	12	0.2
2.	3.35	1.40	1.16	2.64	3	—
2 a.	3.35	—	1.16	2.64	3	0.2
3.	5.27	0.42	0.30	4.75	8	—
3 a.	5.27	—	0.30	4.75	8	0.2
4.	5.31	0.62	0.98	4.75	8	—
4 a.	5.31	—	0.98	4.75	8	0.2

After incubation.			
	Mg. per c.c.		
	H <sub>3</sub> PO <sub>4</sub>	Lactic acid	R.V.
1.	-1.5	+0.59	+2.39
1 a.	-2.5	—	+2.53
2.	-1.48	+1.50	+1.34
2 a.	-3.35	—	+1.71
3.	-1.50	+0.31	+1.01
3 a.	-2.25	—	+1.00
4.	-1.79	+0.64	+0.46
4 a.	-2.88	+0.08	+0.89

Nos. 2 and 2 a were glycogen extracts.

esterification. An accompanying check in lactic acid formation was always observed, which indicates that the ester formed in presence of fluoride is in a stable form.

That fluoride checks hydrolysis of the stable ester is shown from the accompanying curves (Figs. 3 and 4). The graphs in these figures give the time course of esterification and lactic acid production in non-fluoride and fluoride mixtures at 37° and 45°.

At 37° the processes of esterification both in the fluoride and KCl extracts are to be seen, accompanied in the latter by an increase in lactic acid. At a temperature of 45°, after a period of 30 minutes, esterification is checked and, in the KCl extract, hydrolysis of the ester takes place, as shown by an increase in free phosphate and a further small increase in lactic acid, while in presence of fluoride no further change takes place.

The R.V. was found to be slightly higher when the extract was incubated with sodium fluoride which might be due to the check in lactic acid formation, so that the reducing value of the ester was obtained.



The action of fluoride in these extracts will receive further consideration later, especially with regard to the reaction changes associated with ester formation.

KCl extracts lactic acid formation at 37° and 45°  
with and without fluoride

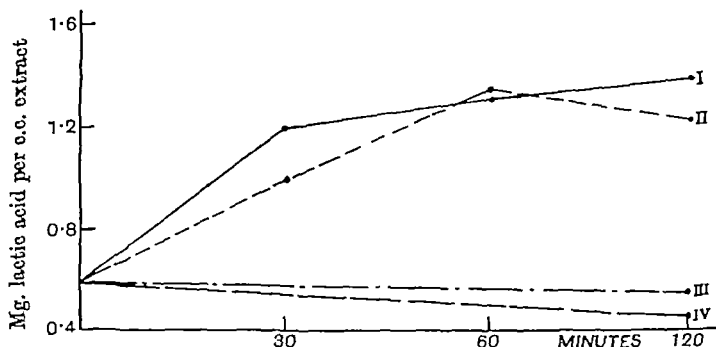


Fig. 3.

I = without fluoride at 45°. II = without fluoride at 37°. III = with fluoride at 45°.  
IV = with fluoride at 37°.

KCl extracts synthesis of  $H_2PO_4$  at 37° and 45°  
with and without fluoride

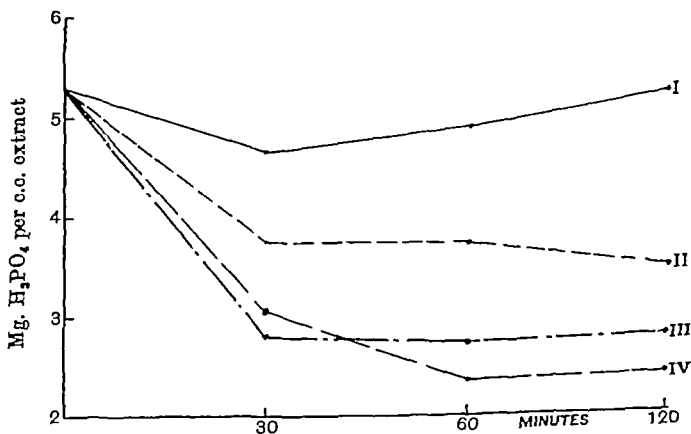


Fig. 4.

I = without fluoride at 45°. II = without fluoride at 37°. III = with fluoride at 45°.  
IV = with fluoride at 37°.

*The influence of temperature on the enzymic changes in the extracts.*

1. *Changes in the extract alone in presence of 0.025 molar NaHCO<sub>3</sub> at 37°, 45° and 50°.*

In most cases for the sake of comparison a portion of the same extract was incubated at a temperature of from 20° to 25°. The results are given in the following table:

TABLE X.

	Temp.	Original values.			Incubation period. Hours	Changes after incubation.		
		Mg. per c.c.				Mg. per c.c.		
		H <sub>2</sub> PO <sub>4</sub>	Lactic acid	R.V.		H <sub>2</sub> PO <sub>4</sub>	Lactic acid	R.V.
1.	22°	0.67	0.85	0.67	3	÷0.12	÷0.20	-0.11
1 a.	37°	0.67	0.85	0.67	1	÷0.13	÷0.20	-0.11
2.	22°	0.68	0.94	0.56	4	÷0.28	÷0.20	-0.21
2 a.	45°	0.68	0.94	0.56	1	÷0.30	÷0.54	-0.32
3.	50°	0.43	0.65	0.43	½	÷0.01	÷0.05	-0.10

From the above table it is seen that the changes which occurred in the extract alone at 37° were similar to those which occurred at 22°. In Nos. 1 and 1 a, which refer to the same extract incubated at 22° and 37° respectively, one hour at 37° has produced as complete a breakdown of the pre-existing ester as three hours at 22°. At a temperature of 45° for one hour the breakdown of hexose phosphate was more complete than at the lower temperature, but at 50° enzymic hydrolysis of the ester was checked.

The reducing sugar has fallen in all cases, due probably to the breakdown of the hexose component of the ester.

2. *Changes in the extracts with added glycogen at different temperatures are shown in the following table:*

TABLE XI.

	Temp.	Original values.			Glycogen addition. Mg. per c.c.
		Mg. per c.c.			
		H <sub>2</sub> PO <sub>4</sub>	Lactic acid	R.V.	
1.	22°	0.67	0.85	0.67	6
1 a.	37°	0.67	0.85	0.67	6
2.	22°	0.68	0.94	0.56	12
2 a.	45°	0.68	0.94	0.65	12
3.	22°	0.67	0.81	0.66	—
3 a.	37°	0.67	0.81	0.66	—

TABLE XI (*contd.*).

	Temp.	Period of incubation. Hours	Changes after incubation.			Glycolysis Esterification
			Mg. per c.c.			
			H <sub>3</sub> PO <sub>4</sub>	Lactic acid	R.V.	
1.	22°	3	-0.25	+0.43	+1.25	1.9
1 a.	37°	1	-0.09	+0.08	+0.98	1.0
2.	22°	4	-0.68	+0.58	+2.82	0.93
2 a.	45°	1	+0.23	+0.26	+5.56	—
3.	22°	4	-0.67	+0.51	+2.64	0.83
3 a.	37°	4	-0.27	+0.23	+3.03	0.96

Nos. 3 and 3 a are glycogen extracts.

When glycogen was added it was evident that the activity of the synthetic but not of the diastatic ferment was diminished at a temperature of 37°, although not completely checked. In the glycogen extract (3 and 3 a) at 22° all the free phosphate disappeared, while at 37° only about one-third was bound. Lactic acid formation at the higher temperature was correspondingly diminished, so that the ratio of glycolysis to esterification remained on an average about the same. At 45° esterification was absent, while lactic acid and phosphoric acid were produced in approximately equimolecular quantities and were obtained therefore from the decomposition of the existing hexose phosphate. It was evident that the diastatic ferment was not injured by the higher temperature, as sugar formation continued and reducing bodies tended to accumulate on account of the check on the further transformation of glycogen.

3. *Changes in the extracts with added phosphate and glycogen at different temperatures* (Table XII).

TABLE XII.

	Temp.	Original values.			Additions.	
		Mg. per c.c.			Mg. per c.c.	
		H <sub>3</sub> PO <sub>4</sub>	Lactic acid	R.V.	Glycogen	H <sub>3</sub> PO <sub>4</sub>
1.	22°	2.94	0.85	0.67	6	2.26
1 a.	37°	2.94	0.85	0.67	6	2.26
2.	22°	11.52	0.99	0.46	12	10.6
2 a.	37°	11.52	0.99	0.46	12	10.6
3.	22°	11.51	1.14	0.82	12	10.6
3 a.	37°	11.51	1.14	0.82	12	10.6
4.	22°	5.88	0.94	0.56	12	5.3
4 a.	45°	5.88	0.94	0.56	12	5.3
5.	26°	5.60	0.65	0.43	12	5.18
5 a.	50°	5.60	0.65	0.43	12	5.18

TABLE XII (contd.).

		Changes after incubation.				Glycolysis Esterification
		Period of incubation Hours	Mg. per c.c.			
Temp.			H <sub>3</sub> PO <sub>4</sub>	Lactic acid	R.V.	
1.	22°	3	-1.42	÷ 0.21	÷ 0.92	0.16
1 a.	37°	1	-0.99	÷ 0.71	÷ 2.05	0.77
2.	22°	4	-6.91	÷ 2.80	÷ 1.71	0.45
2 a.	37°	2	-6.44	÷ 3.49	÷ 1.66	0.59
3.	22°	4	-8.42	÷ 3.33	÷ 2.38	0.43
3 a.	37°	4	-7.88	÷ 3.69	÷ 3.36	0.51
4.	22°	4	-4.3	÷ 1.5	÷ 2.64	0.39
4 a.	45°	1	-1.85	-1.25	÷ 4.38	0.73
5.	26°	3	-1.5	÷ 0.59	÷ 2.39	0.43
5 a.	50°	4	÷ 0.1	÷ 0.12	—	—

When both phosphate and glycogen were added to the extracts, and the mixtures incubated at a temperature of 37°. synthesis was again seen to be diminished when compared with the same extract kept at a lower temperature, but in all cases there was a greater increase in lactic acid than in the same extract kept at 22°. When glycogen alone was present there was a marked impairment in synthesis and glycolysis, while when both phosphate and glycogen were added synthesis was only slightly impaired, and there was, if anything, an increased production of lactic acid at the higher temperature. This increase in lactic acid, accompanied by a decrease in the quantity of ester present, may be due to a tendency for the stable ester which has been built up to break down more readily at 37° than at 22°. On the whole, whether glycogen alone or whether both phosphate and glycogen be added, the amount of bound phosphate in the mixture at the end of incubation at 37° was less than at the lower temperature. The diastatic action as seen from the R.V. was unaffected by the higher temperature.

When the extract was incubated at 45° with phosphate and glycogen some synthesis could still take place, although, as has been pointed out, there was no esterification when glycogen alone was added. Lactic acid can still be produced at 45°, although not to the same extent as at the lower temperature. At 50° esterification was completely checked even when phosphate was added, and at the same time enzymic hydrolysis of the pre-existing ester was interfered with.

*The reaction changes accompanying the enzymic processes.*

These changes may be more satisfactorily studied in extracts prepared by Meyerhof's method than in the expressed juice, because the former may be made so rapidly that the protein and the lactic acid are

at quite low values, while in the latter these may attain fairly high concentrations. The extracts also contain only a small amount of their phosphate bound either in an unstable or in a stable form.

After mixture of such extracts with phosphate or phosphate plus glycogen, the enzymic processes may be examined under fairly simple conditions. In all cases it is necessary to study the reaction and the chemical changes concurrently.

In the first place these changes may be followed at various time intervals, and the following example may be taken as more or less typical of the general course of the reaction in the presence and in the absence of fluoride.

The following mixtures were incubated at 25°:

B. 35 c.c. of 0.15 *N* KCl extract of rabbit's muscle were mixed with 35 c.c. of 0.05 molar  $\text{Na}_2\text{HPO}_4$  plus 0.24 gm. glycogen.

C. 35 c.c. of the same extract mixed with 35 c.c. 0.05 molar phosphate containing 0.14 gm. sodium fluoride and 0.24 gm. glycogen.

### Results.

TABLE XIII.

#### (1) Reaction changes.

B.				C.			
Time (min.)	pH	$(\text{H}^+)10^{-7}$	$\Delta\text{H}^+/\Delta t$	Time (min.)	pH	$(\text{H}^+)10^{-7}$	$\Delta\text{H}^+/\Delta t$
0	7.11	0.769	—	0	7.11	0.769	—
10	6.89	1.265	0.049	10	6.95	1.100	0.033
20	6.82	1.515	0.025	20	6.85	1.400	0.030
30	6.79	1.610	0.009	30	6.79	1.610	0.021
40	6.76	1.705	0.009	40	6.76	1.705	0.009
120	6.60	2.495	0.009	120	6.55	2.810	0.013

#### (2) Chemical changes.

B.				C.			
Time (min.)	$\text{H}_2\text{PO}_4$ (free) (milli- mols)	$\Delta\text{H}_2\text{PO}_4$ (ester) $\Delta t$	Lactic acid (milli- mols)	Time (min.)	$\text{H}_2\text{PO}_4$ (free) (milli- mols)	$\Delta\text{H}_2\text{PO}_4$ (ester) $\Delta t$	Lactic acid (milli- mols)
0	32.6	—	10.2	0	30.9	—	8.4
10	27.7	0.49	11.1	10	24.4	0.65	9.3
20	25.0	0.27	12.6	20	20.8	0.36	8.0
30	24.2	0.08	—	30	12.7	0.81	9.3
120	24.3	—	16.9	120	11.9	0.009	11.0

The variations in the amount of lactic acid in *C* are (probably apart from the last value) within the range of experimental error in a fluoride mixture.

*Summary of Results.*

1. The reaction changes in the presence and in the absence of fluoride are essentially of the same order. In this particular experiment the rise in acidity in *B* is slightly more marked in the first 20 minutes. At 30 and at 40 minutes the reactions are identical, but at the end of the two hours' period the fluoride is slightly the more acid mixture.

2. The chemical changes in the two mixtures are different. In both esterification is the main change in the first 20 minutes. as even in *B* during this time there is but little lactic acid formation. The fall in free phosphate (the formation of the ester) is more rapid in *C* than *B*. As in *B* during the first 10 minutes 0.49 millimol free  $\text{H}_3\text{PO}_4$  is disappearing per minute, and in the next 10 minutes 0.27 millimol, while but little lactic acid is produced, it is probable that the ester which is being formed is the monophosphoric acid ester, and if this be the case during this initial period as much or more hexose may be esterified in *B* than in *C*, depending upon the relative amounts of the mono- and di-ester in each case. During the last 90 minutes, in *B* the free phosphate remains at a steady level while the lactic acid is increasing. so that the process during this period is probably one in which the mono- is changed into the di-ester. If we take approximately 4.3 millimols of lactic acid as the production during this period this would necessitate the transformation of 4.3 millimols of the mono- into 2.15 of the di-ester with the setting free of 2.15 millimols of reactive sugar to form the lactic acid. In this mixture during the first 20 minutes 7.6 millimols  $\text{H}_3\text{PO}_4$  have been bound, with 7.6 millimols of hexose (presupposing mono-ester formation), and of these 4.3 have undergone the further change.

In *C* in the two-hour period, during which acidity has risen to a slightly higher level than in *B*, 19 millimols  $\text{H}_3\text{PO}_4$  have been bound. In the last 90 minutes when esterification has been very slight. it appears probable that there has been lactic acid formation, otherwise it would be difficult to account for the continued rise in acidity.

It is evident that even with no lactic acid production. esterification alone is associated with a rise in acidity. as has been shown by Meyerhof and his co-workers. As the esters which have been examined by Meyerhof, Lohmann and Suranyi have higher dissociation constants than those of orthophosphoric acid. it is natural to suppose that the rise in acidity in the incubated mixtures is due to the formation of such acids. Further information regarding the probable dissociation constants of these esters in the zone of reaction examined may be obtained from the study of electrometric titration curves.

Mixtures *B* and *C* were therefore titrated and the *pH* changes determined after the addition of known amounts of base with the following results.

TABLE XIV.

Incubated mixture <i>B</i> .				Incubated mixture <i>C</i> .			
B (base addition molar)	<i>pH</i>	$\Delta B/\Delta pH$	Mean <i>pH</i>	<i>B</i>	<i>pH</i>	$\Delta B/\Delta pH$	Mean <i>pH</i>
—	6.60	—	—	—	6.55	—	—
0.004	6.820	0.0184	6.71	0.008	6.94	0.0205	6.74
0.0075	7.014	0.0180	6.91	0.012	7.28	0.0115	7.11

A 0.0326 molar phosphate solution (equal to the  $H_3PO_4$  concentration of *B* and *C*) gave the following values for approximately the same reaction zones.

<i>B</i>	<i>pH</i>	$\Delta B/\Delta pH$	Mean <i>pH</i>
—	6.50	—	—
0.0055	6.80	0.0183	6.65
0.0094	7.00	0.0195	6.90
0.0143	7.30	0.0163	7.15

When these three mixtures are compared, it is evident that *B* behaves very much as an inorganic phosphate mixture with a slightly lower B.V. between 6.8 and 7.0 *pH*. Mixture *C* shows a marked diminution in B.V. on the alkaline side of *pH* 6.9 and a raised B.V. on the acid side, that is to say, an acid or acids have appeared replacing in part the orthophosphate, and these have their maximal buffering capacity on the acid side of the inorganic salt solution's maximal buffering zone.

It is important to note that even at the beginning of the process esterification in the presence of fluoride is apparently more rapid than in its absence. This possibly may be due to the fact that the mono-ester which is primarily produced in both cases is in a more stable form in the presence of fluoride, so that by the method of estimation of the free phosphate (E. or F. S.) some at least of the less stable form may be broken down in the chloride and not in the fluoride mixture. It is to be noted that although in the presence of fluoride 6.5 millimols of  $H_3PO_4$  are bound in the first 10 minutes, and 4.9 in its absence, while lactic acid production is extremely small in both, the acid change in *B* is slightly greater than in *C*.

It is possible that during the continuous esterification in fluoride both the initial mono-ester and di-ester are more stable than in non-fluoride mixtures, and that even at the close of the incubation period the mixture contains in stable form both mono- and di-esters. The buffering capacity of fluoride mixtures at the close of incubation usually points to the presence of buffering salts with acids of slightly higher

dissociation constant than in chloride mixtures. The inorganic phosphates may in such a case be replaced by a mixture of stable mono- or di-esters, so that the B.V. in the neighbourhood of the neutral point falls markedly, while on the acid side it shows an increase. Unfortunately in these particular experiments the buffering in the more acid zone was not determined. Experiments dealing with this point will be described later on.

*The reaction changes accompanying the enzymic changes  
at 37° and 47°.*

The changes which occur at higher temperatures will now be given for comparison with the preceding series.

Two sets of mixtures were examined, one at 37° and the other at 47°. the same rabbit's muscle extract being used in both. First series at 37°.

B. 30 c.c. extract. 30 c.c. 0.05 molar,  $\text{Na}_2\text{HPO}_4$ , 240 mg. glycogen.

C. The same but containing in addition 0.12 gm. fluoride.

D. 16 c.c. extract, 16 c.c. phosphate (0.05 M.). 64 mg. glucose.

E. The same as D, with 64 mg. NaF added.

The second series was the same, and was incubated at 47°.

In the first place mixtures D and E both at 37° and 47° showed no change either in reaction or in free phosphate or lactic acid values during the incubation process, so that these need not be referred to. One may simply draw the conclusion that when the muscle extract in the presence of glycogen and phosphate produces definite chemical changes, there are always the characteristic accompanying reaction shifts. On the other hand, if such an extract produces no chemical changes, as for example in the cases above mentioned, when glucose was added. the reaction remains unaltered.

TABLE XV. Reaction and chemical changes.

B.				B.			
Time (min.)	At 37°		Lactic acid (mmol)	Time (min.)	At 47°		Lactic acid (mmol)
	pH	$\text{H}_2\text{PO}_4$ (mmol)			pH	$\text{H}_2\text{PO}_4$ (mmol)	
0	7.64	27.06	3.44	0	7.64	27.06	3.40
60	6.89	19.0	7.60	30	7.13	23.87	6.69
				120	6.79	26.73	8.00
C.				C.			
Time (min.)	(Fluoride) at 37°		Lactic acid	Time (min.)	At 47°		Lactic acid
	pH	$\text{H}_2\text{PO}_4$			pH	$\text{H}_2\text{PO}_4$	
0	7.63	25.66	3.44	0	7.63	27.06	3.4
60	6.76	12.19	3.88	30	6.62	14.28	—
				120	6.89	14.34	3.4



*Changes in one hour at 37° compared with those in half an hour at 47°.*

In the absence of fluoride at both temperatures there is esterification along with lactic acid production, and the accompanying rise in acidity. The reaction change is much greater at the lower temperature within a period of an hour than at the higher in 30 minutes, and the cause of this is undoubtedly the larger amount of ester present. Calculating for di-ester formation, 4 millimols hexose have been esterified for 2.08 glycolysed, and at the higher temperature 1.6 esterified and 1.6 glycolysed.

In the presence of fluoride at the lower temperature the reaction change is nearly the same as in its absence and 6.73 millimols hexose have been esterified, being rather more than the total hexose changed in the absence of fluoride. At the higher temperature in the presence of fluoride in 30 minutes the reaction swing and the esterification change are of the same order as at the lower temperature.

In the absence of fluoride at the higher temperature within the last  $1\frac{1}{2}$  hours there has been a rise in acidity accompanied by the setting free of 1.43 millimols hexose and the glycolysis of 0.7 millimol.

It appears therefore that in the first 30 minutes equimolecular esterification and glycolysis took place, and in the succeeding 90 minutes of the 1.6 millimols of hexose which were esterified 1.43 millimols were again set free, and of this quantity 50 p.c. was broken down to lactic acid.

*The B.V. of unincubated and incubated ferment mixtures.*

Reference has already been made to the changes in B.V. produced by incubation of the muscle juice, of extracts of muscle fibres from which the juice has been expressed and of saline extracts of the cooled minced muscle in the presence of phosphate and glycogen or starch. It is advisable, however, to deal with this subject more fully and to consider the influence of the protein present on the buffering capacities of the mixtures.

The mixtures in which the protein must play a subordinate rôle are those in which the colloidal content is low and in which the main buffering is due to the added free phosphate, and these may most readily be obtained by the method of extraction employed by Meyerhof and his co-workers. As Meyer(8) has shown, the protein content of such extracts may be brought to an extremely low level by suitable methods of precipitation and elution, finally employing Willstätter's procedures for the purification.

In our hands, however, extracts prepared in such a way as to bring down the protein to a very low level (adsorption and the elution of  $\text{Al}(\text{OH})_2$  precipitates) have not proved very suitable for electrometric titration, and so attention will be solely directed in this paper to the B.V. of mixtures which have a protein nitrogen content approximately 0.05 p.c. Such protein holding mixtures, even after incubation, may have the larger part of their protein removed by rapid steaming without altering to anything but a very slight extent the relative amounts of free and bound (stable) hexose phosphate.

Unincubated and incubated mixtures may therefore be titrated before and after removal of the major part of the protein. It is fully recognised that such a procedure as heat coagulation of protein may give rise to the breaking down of certain linkages, physical or chemical, which may alter the B.V., but one may at least compare protein holding unincubated with similar incubated mixtures, and also deproteinised unincubated with deproteinised incubated.

It is essential, naturally, to know the composition of the various mixtures as regards constituents which will affect the buffering capacity, and therefore along with the electrometric titration values there must be an accompanying analysis of the mixtures.

An example will be fully dealt with in order that the procedure generally adopted may be understood.

Equal volumes of the saline extract and 0.05 M.  $\text{Na}_2\text{HPO}_4$  were mixed, and 8 mg. glycogen per c.c. extract added. To one portion fluoride was added up to 0.1 M. concentration.

Portions of the mixture, with and without fluoride, were placed in the thermostat and incubated for  $2\frac{1}{2}$  hours at  $30^\circ$ .

Another measured portion was deproteinised by steaming, the steam issuing from a multi-perforated glass bulb on a tube connected with a flask in which water was boiling. The mixture was raised to boiling point in about 15 seconds, and was then rapidly cooled, the increase in volume noted, so that the protein holding unsteamed mixture could be diluted to the same extent. The mixture was then filtered.

The reactions of the unsteamed and steamed portions were determined, and both were analysed.

To obtain the B.V. of the two unincubated mixtures (with and without protein) the usual electrometric titration was carried out.

After incubation for  $2\frac{1}{2}$  hours, portions of the mixtures, with and without fluoride, were deproteinised in the same way, analysed and their B.V. determined.

All the phosphate determinations were made by the F. S. and lactic acid by the Friedemann, Cotonio and Shaffer methods (9).

(1) *The unincubated protein holding mixture.*

pH 7.14.

Protein nitrogen 0.066 p.c.

Free phosphate 0.0215 M. Hexose phosphate 0.0013 M.

Lactic acid 0.0053 „

(2) *The same after deproteinisation.*

pH 7.18.

Free phosphate 0.0209 M. Hexose phosphate 0.001 M.

Lactic acid 0.0053 „

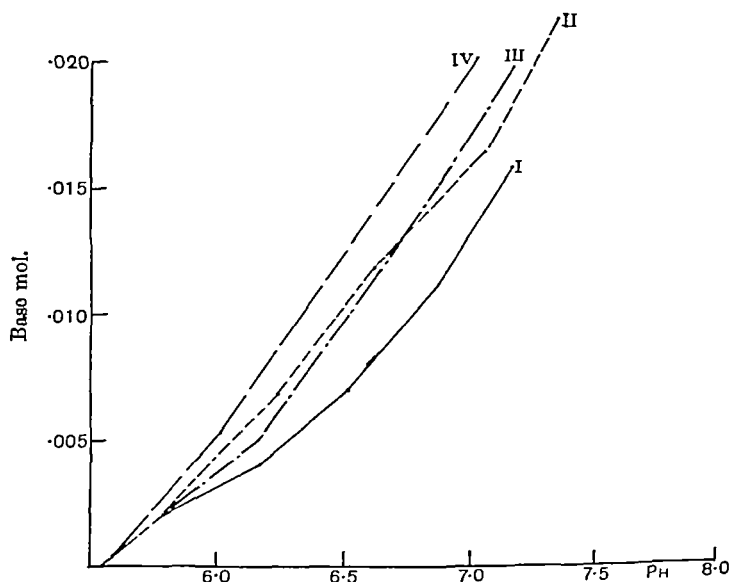


Fig. 5. Buffer graphs.

I = 0.022 molar phosphate.

II = B after incubation  $2\frac{1}{2}$  hrs. at  $30^{\circ}$

III = B and C before incubation

IV = C after incubation

} containing protein.

*B.V. of mixtures before incubation (Table XVI).*

In all cases these are given in terms of base addition (molar), although the titration starting from the alkaline reaction was carried out by acid addition.

4 c.c. of the mixture were taken, 0.1 to 0.4 c.c. M./5 acid added in steps, in each case the volume being made up to 4.5 c.c. by the addition of water. The concentrations given above have been corrected for this degree of dilution.

Figs. 5 and 6 give the buffer graphs of the unincubated and incubated mixtures, the base addition for a range of  $pH$  from 5.5 to 7.5 being given.

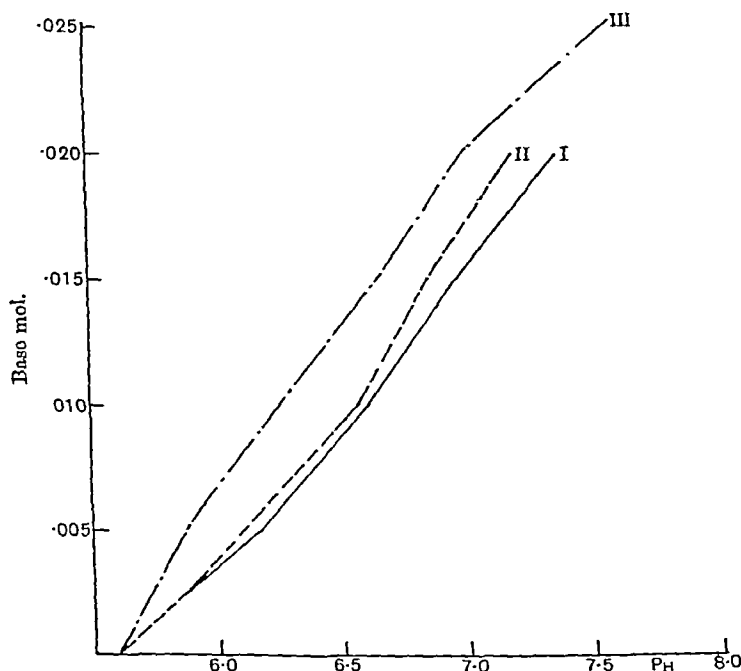


Fig. 6. Buffer graphs.

I = B' after 2½ hrs. incubation }  
 II = B before incubation } deproteinised.  
 III = C' after 2½ hrs. incubation }

TABLE XVI. B.V. of mixtures before incubation.

B	(1) With protein.			B	(2) Without protein.		
	pH	$\Delta B/\Delta pH$	Mean pH		pH	$\Delta B/\Delta pH$	Mean pH
0.0045	5.55	—	—	—	5.88	—	—
0.0080	6.16	0.0074	5.85	0.0067	6.56	0.0098	6.22
0.0135	6.50	0.0132	6.33	0.0112	6.84	0.0160	6.70
0.0180	6.84	0.0132	6.67	0.0157	7.18	0.0132	7.01
	7.14	0.0150	6.90				

All the phosphate determinations were made by the F. S. and lactic acid by the Friedemann, Cotonio and Shaffer methods (9).

(1) *The unincubated protein holding mixture.*

pH 7.14.

Protein nitrogen 0.066 p.c.

Free phosphate 0.0215 M. Hexose phosphate 0.0013 M.

Lactic acid 0.0053 „

(2) *The same after deproteinisation.*

pH 7.18.

Free phosphate 0.0209 M. Hexose phosphate 0.001 M.

Lactic acid 0.0053 „

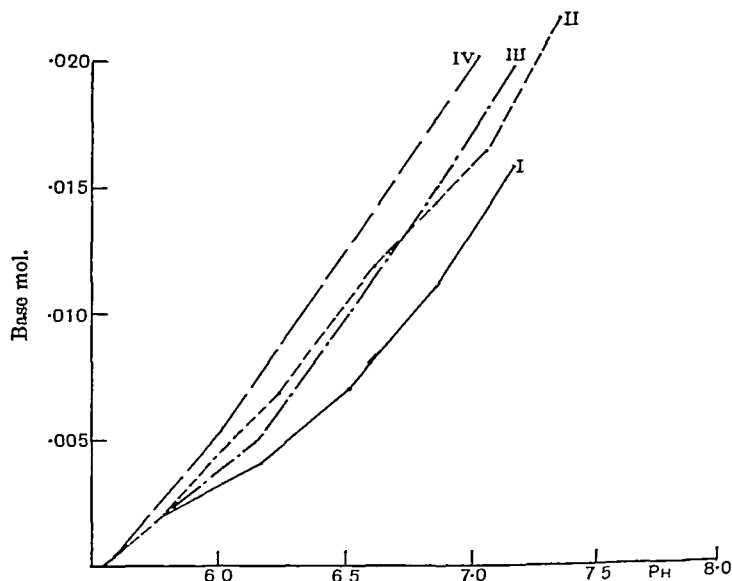


Fig. 5. Buffer graphs.

I = 0.022 molar phosphate.

II = B after incubation 2½ hrs. at 30°  
 III = B and C before incubation  
 IV = C after incubation

} containing protein.

*B.V. of mixtures before incubation (Table XVI).*

In all cases these are given in terms of base addition (molar), although the titration starting from the alkaline reaction was carried out by acid addition.

4 c.c. of the mixture were taken. 0.1 to 0.4 c.c.  $M/5$  acid added in steps, in each case the volume being made up to 4.5 c.c. by the addition of water. The concentrations given above have been corrected for this degree of dilution.

Figs. 5 and 6 give the buffer graphs of the unincubated and incubated mixtures, the base addition for a range of pH from 5.5 to 7.5 being given.

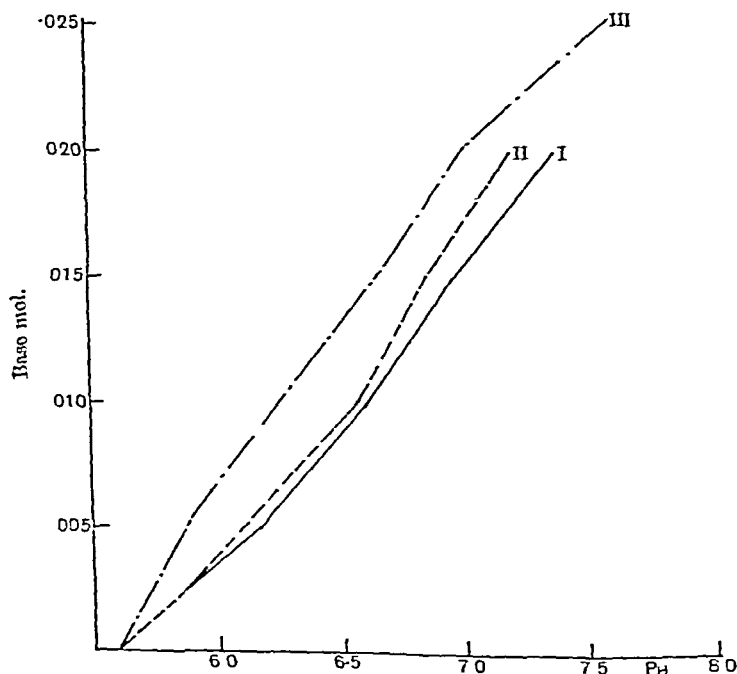


Fig. 6. Buffer graphs.

I =  $B'$  after  $2\frac{1}{2}$  hrs. incubation  
 II =  $B$  before incubation  
 III =  $C'$  after  $2\frac{1}{2}$  hrs. incubation } deproteinised.

TABLE XVI. B.V. of mixtures before incubation.

(1) With protein.				(2) Without protein.			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
0.0045	5.55	—	—	0.0067	5.88	—	—
0.0090	6.16	0.0074	5.85	0.0067	6.56	0.0098	6.22
0.0135	6.50	0.0132	6.33	0.0112	6.84	0.0160	6.70
0.0180	6.84	0.0132	6.67	0.0157	7.18	0.0132	7.01
	7.14	0.0150	6.99				

B.V. after incubation in absence of fluoride for 2½ hours at 30° (Table XVII).

(1) The protein holding mixture.				(2) Deproteinised.			
pH 6.59				pH 6.60			
Phosphate free	.....	0.0151 m.		Phosphate free	.....	0.0153 m.	
" labile	.....	0.0024 m.		" labile	.....	0.0019 m.	
" ester	.....	0.005 m.		" ester	.....	0.0043 m.	
Lactic acid	.....	0.0100 m.		Lactic acid	.....	0.0100 m.	

TABLE XVII. B.V. of incubated mixtures.

(1)				(2)			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
—	5.77	—	—	—	5.59	—	—
0.0045	6.23	0.0093	6.00	0.0045	6.17	0.0077	5.83
0.0090	6.59	0.0125	6.41	0.0090	6.60	0.0104	6.53
0.0135	7.06	0.0095	6.82	0.0135	6.95	0.0129	6.73
				0.0180	7.36	0.0109	7.15

B.V. after incubation in presence of fluoride for 2½ hours at 30° (Table XVIII).

(1) The protein holding mixture.				(2) Deproteinised.			
pH 6.67				pH 6.65			
Phosphate free	.....	0.0129 m.		Phosphate free	.....	0.0129 m.	
" labile	.....	—		" ester	.....	0.0096 m.	
" ester	.....	0.0096 m.		Lactic acid	.....	0.0050 m.	
Lactic acid	.....	0.0056 m.					

TABLE XVIII. B.V. of incubated fluoride mixtures.

(1)				(2)			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
—	5.59	—	—	—	5.61	—	—
0.0045	6.01	0.0107	5.80	0.0045	5.89	0.0160	5.75
0.0090	6.34	0.0137	6.17	0.0090	6.26	0.0122	6.07
0.0135	6.67	0.0137	6.50	0.0135	6.65	0.0115	6.45
0.0180	7.00	0.0137	6.83	0.0180	7.00	0.0129	6.82
				0.0225	7.57	0.0079	7.28

The mixtures before and after incubation with and without protein and in the slightly diluted state used for titration contained in all cases practically the same total amount of phosphate 0.020 to 0.025 molar. Prior to incubation there was only a very small amount in ester form, as determined by hydrolysis in bicarbonate solution at 45°. The lactic acid content was in all unincubated mixtures 0.005 molar.

After incubation in the absence of fluoride, the protein holding mixtures contained 67.3 p.c. free phosphate, 22.2 p.c. as stable hexose phosphate and 10.5 p.c. in labile form.

The deproteinised incubated mixture 71.8 p.c. was in the free form,

20.1 p.c. in labile form and 8.7 p.c. in ester form.

In all cases these mixtures showed the same increase, namely, 0.0047 molar, in the titration starting with the fluoride mixture 57.3 p.c. was free and 42.7 p.c. was no increment in lactic acid.

Comparison of the buffering of the protein holding mixtures before and after incubation in the presence and in the absence of fluoride.

(1) Unincubated: Maximal buffering at a mean  $pH$  of approximately 7, remaining at a lower but steady value between  $pH$  6.16 and 6.76 and falling to a low value at a mean  $pH$  5.85.

(2) Incubated in absence of fluoride: Maximal buffering at mean  $pH$  of 6.41.

(3) Incubated in presence of fluoride: Maximal buffering steady over a wide zone from  $pH$  6 to 7. When the  $pK'$  values of two acids are a  $pH$  unit apart and in equal concentration the overlapping of the buffer effects should give rise nearly to a constant B.V. in the intervening zone. In such a mixture, however, as muscle extract with other bodies present (e.g. protein, etc.), such an effect may in part be brought about by other agencies.

Comparison of the B.V. of the mixtures deproteinised by steaming.

(1) Unincubated: Maximal buffering at  $pH$  (mean) 6.7.

(2) Incubated in absence of fluoride: Maximal buffering at a mean  $pH$  6.78, but the B.V. is distinctly lower than in the deproteinised unincubated mixture in this region, also the B.V. on the alkaline side is lower and on the acid side slightly higher than in the latter.

(3) Incubated in presence of fluoride: Maximal B.V. much more on the acid side, viz.,  $pH$  5.75; then, after falling at  $pH$  6, it remains fairly steady to  $pH$  7, and then sinks to a low value on the alkaline side.

With ester formation there is therefore an alteration in the zone of maximal buffering, and if the proportion in ester form be sufficiently high there is a distinct fall in the B.V. on the alkaline side and a rise on the acid side when compared with unincubated mixtures.

So long as the protein content is low, it is probably better to determine the buffering capacity of the mixtures when these have not been subjected to such a process as steaming for the removal of the protein, even although the proportions of free to bound phosphates be almost unaffected by this procedure.

Two additional experiments may be described which enable a comparison to be made between an incubated mixture with a high content of lactic acid and a small amount of ester still not broken down and one which has also a high content of lactic acid and in which practically all the ester is broken down. For comparison, in each experiment a portion was incubated with fluoride so that the influence of esterification on reaction and on the B.V. in various  $pH$  zones might also be studied.



B.V. after incubation in absence of fluoride for 2½ hours at 30° (Table XVII).

(1) The protein holding mixture.

pH 6.59	
Phosphate free .....	0.0151 m.
„ labile.....	0.0024 m.
„ ester .....	0.005 m.
Lactic acid .....	0.0100 m.

(2) Deproteinised.

pH 6.60	
Phosphate free .....	0.0158 m.
„ labile.....	0.0019 m.
„ ester .....	0.0043 m.
Lactic acid .....	0.0100 m.

TABLE XVII. B.V. of incubated mixtures.

(1)				(2)			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
—	5.77	—	—	—	5.59	—	—
0.0045	6.23	0.0098	6.00	0.0045	6.17	0.0077	5.88
0.0090	6.59	0.0125	6.41	0.0090	6.60	0.0104	6.39
0.0135	7.06	0.0095	6.82	0.0135	6.95	0.0129	6.78
				0.0180	7.36	0.0109	7.15

B.V. after incubation in presence of fluoride for 2½ hours at 30° (Table XVIII).

(1) The protein holding mixture.

pH 6.67	
Phosphate free .....	0.0129 m.
„ labile.....	—
„ ester .....	0.0096 m.
Lactic acid .....	0.0056 m.

(2) Deproteinised.

pH 6.65	
Phosphate free .....	0.0129 m.
„ ester .....	0.0096 m.
Lactic acid .....	0.0050 m.

TABLE XVIII. B.V. of incubated fluoride mixtures.

(1)				(2)			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
—	5.59	—	—	—	5.61	—	—
0.0045	6.01	0.0107	5.80	0.0045	5.89	0.0160	5.75
0.0090	6.34	0.0137	6.17	0.0090	6.26	0.0122	6.07
0.0135	6.67	0.0137	6.50	0.0135	6.65	0.0115	6.45
0.0180	7.00	0.0137	6.83	0.0180	7.00	0.0129	6.82
				0.0225	7.57	0.0079	7.28

The mixtures before and after incubation with and without protein and in the slightly diluted state used for titration contained in all cases practically the same total amount of phosphate 0.020 to 0.025 molar. Prior to incubation there was only a very small amount in ester form, as determined by hydrolysis in bicarbonate solution at 45°. The lactic acid content was in all unincubated mixtures 0.005 molar.

After incubation in the absence of fluoride, the protein holding mixtures contained 67.3 p.c. free phosphate, 22.2 p.c. as stable hexose phosphate and 10.5 p.c. in labile form.

The deproteinised incubated mixture 71.8 p.c. was in the free form, 20.2 p.c. in stable hexose phosphate form and 8.7 p.c. in labile form.

In all cases these mixtures showed the same increase, namely, 0.0047 molar. In the titration starting with the fluoride mixture 57.3 p.c. was free and 42.7 p.c. was in ester form. There was no increment in lactic acid.

Comparison of the buffering of the protein holding mixtures before and after incubation in the presence and in the absence of fluoride.

(1) Unincubated: Maximal buffering at a mean  $pH$  of approximately 7, remaining at a lower but steady value between  $pH$  6.16 and 6.76 and falling to a low value at a mean  $pH$  5.85.

(2) Incubated in absence of fluoride: Maximal buffering at mean  $pH$  of 6.41.

(3) Incubated in presence of fluoride: Maximal buffering steady over a wide zone from  $pH$  6 to 7. When the  $pK'$  values of two acids are a  $pH$  unit apart and in equal concentration the overlapping of the buffer effects should give rise nearly to a constant B.V. in the intervening zone. In such a mixture, however, as muscle extract with other bodies present (e.g. protein, etc.), such an effect may in part be brought about by other agencies.

Comparison of the B.V. of the mixtures deproteinised by steaming.

(1) Unincubated: Maximal buffering at  $pH$  (mean) 6.7.

(2) Incubated in absence of fluoride: Maximal buffering at a mean  $pH$  6.78, but the B.V. is distinctly lower than in the deproteinised unincubated mixture in this region, also the B.V. on the alkaline side is lower and on the acid side slightly higher than in the latter.

(3) Incubated in presence of fluoride: Maximal B.V. much more on the acid side, viz.,  $pH$  5.75; then, after falling at  $pH$  6, it remains fairly steady to  $pH$  7, and then sinks to a low value on the alkaline side.

With ester formation there is therefore an alteration in the zone of maximal buffering, and if the proportion in ester form be sufficiently high there is a distinct fall in the B.V. on the alkaline side and a rise on the acid side when compared with unincubated mixtures.

So long as the protein content is low, it is probably better to determine the buffering capacity of the mixtures when these have not been subjected to such a process as steaming for the removal of the protein, even although the proportions of free to bound phosphates be almost unaffected by this procedure.

Two additional experiments may be described which enable a comparison to be made between an incubated mixture with a high content of lactic acid and a small amount of ester still not broken down and one which has also a high content of lactic acid and in which practically all the ester is broken down. For comparison, in each experiment a portion was incubated with fluoride so that the influence of esterification on reaction and on the B.V. in various  $pH$  zones might also be studied.

A mixture (*pH* 7.06) of the following composition was incubated for three hours at 30°, 4 mg. glycogen being added per c.c. of the mixture. The composition was as follows:

	Before incubation. <i>B</i>	After incubation:	
		<i>B'</i> Without fluoride	<i>C'</i> With fluoride
Free phosphate	0.0227 m.	0.0210	0.0140
Labile „	0.0023 m.	0.0007	0
Ester „	0.0045 m.	0.0078	0.0155
Lactic acid	0.0084 m.	0.0163	0.0072
Protein	0.6 %		

After incubation in the absence of fluoride, the reaction was *pH* 6.30 and in its presence 6.33.

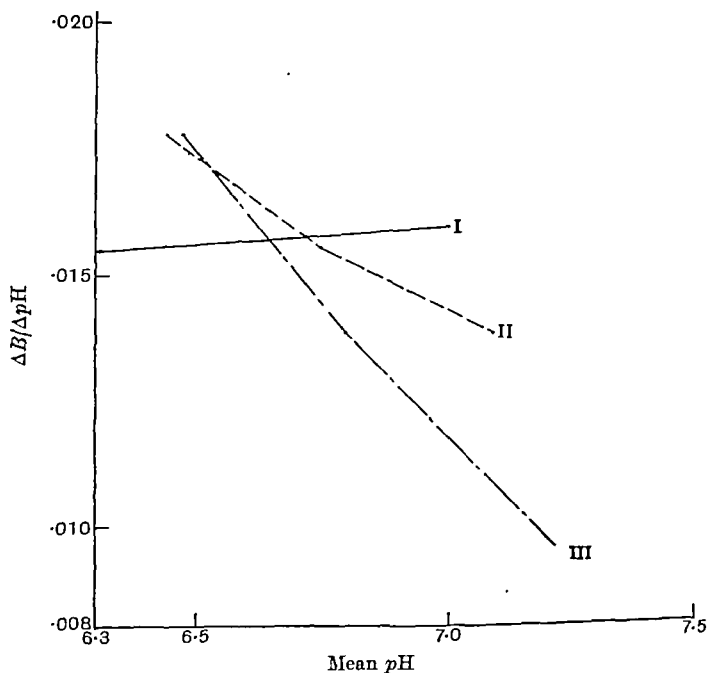


Fig. 7.

I = *B* before incubation.    II = *B* after incubation.    III = *C* after incubation.

TABLE XIX. Electrometric titration. (Fig. 7.)

<i>B.</i>				<i>B'.</i>			
<i>B</i>	<i>pH</i>	$\Delta B/\Delta pH$	Mean <i>pH</i>	<i>B</i>	<i>pH</i>	$\Delta B/\Delta pH$	Mean <i>pH</i>
—	6.17	—	—	—	6.30	—	—
0.0045	6.46	0.0155	6.31	0.0048	6.58	0.0178	6.44
0.0092	6.76	0.0157	6.61	0.0095	6.90	0.0156	6.74
0.0140	7.06	0.0160	6.91	0.0140	7.26	0.0139	7.08

TABLE XIX (*contd.*).

C'.	B	pH	$\Delta B/\Delta pH$	Mean pH
	0	6.33	—	—
	0.005	6.61	0.0178	6.47
	0.010	6.97	0.0139	6.79
	0.015	7.50	0.0094	7.23

The change in reaction produced by incubation in the presence and in the absence of fluoride was almost the same. In the former this was accompanied by the amount of esterification noted above with only very slight lactic acid production, in the latter the mixture at the end showed a marked increase in lactic acid (about 0.01 molar). with a slight increase in the hexose phosphate above the unincubated value.

All three mixtures contained about 0.6 p.c. protein.

In the unincubated mixture which contained a rather higher hexose phosphate content than was commonly met with, the buffering from pH 6.17 to 7.06 remained fairly steady. being more regular and of a slightly higher value than that shown by a corresponding inorganic phosphate solution. The B.V. rose slightly towards the neutral point. In the incubated mixtures, with and without fluoride, the B.V. was higher on the acid side, falling more rapidly towards the alkaline side in the fluoride mixture. This higher buffer capacity on the acid side is due partly to the protein which has parted with base during the acid change on incubation and partly to the presence of an acid or acids with dissociation constants corresponding to the mean pH in this region. The significant differences between the fluoride and the other incubated mixture towards the alkaline side are due to the great fall in free phosphate in the former so that in the zone of maximal buffering (pH 6.8 to 7.0) of  $H_3PO_4$  the B.V. proportionately falls. Throughout the whole range, however, protein or constituents other than phosphate are raising the B.V., for example, at the mean pH 6.47 in the fluoride incubated mixture probably about 0.005 of the B.V. is due to buffers other than the free and bound phosphates.

The pH zone covered by electrometric titration in the preceding experiments did not reach sufficiently to the acid side to discover when the B.V. began to fall, which was to be expected in any case where there was but little ester present to keep the value up. In the next example, therefore, a wider zone was tested and incubation was allowed to continue until practically all the ester in the non-fluoride specimen had broken down.

A mixture of muscle extract with phosphate, containing as before

4 mg. glycogen per c.c., was incubated for  $4\frac{1}{2}$  hours at  $30^\circ$ , one portion with fluoride and the other without. The reaction of the mixture before incubation (B) was pH 7.22, after incubation in absence of fluoride (B') pH 6.40 and in its presence (C') pH 6.39.

The composition of these mixtures was as follows:

	(B)	(B')	(C')
Free phosphate	0.0247 m.	0.0234 m.	0.0139 m.
Labile "	0.0016 m.	A trace	Absent
Hexose "	0.0030 m.	0.0040 m.	0.0141 m.
Lactic acid	0.0047 m.	0.0131 m.	0.0053 m.
Protein	0.5%		

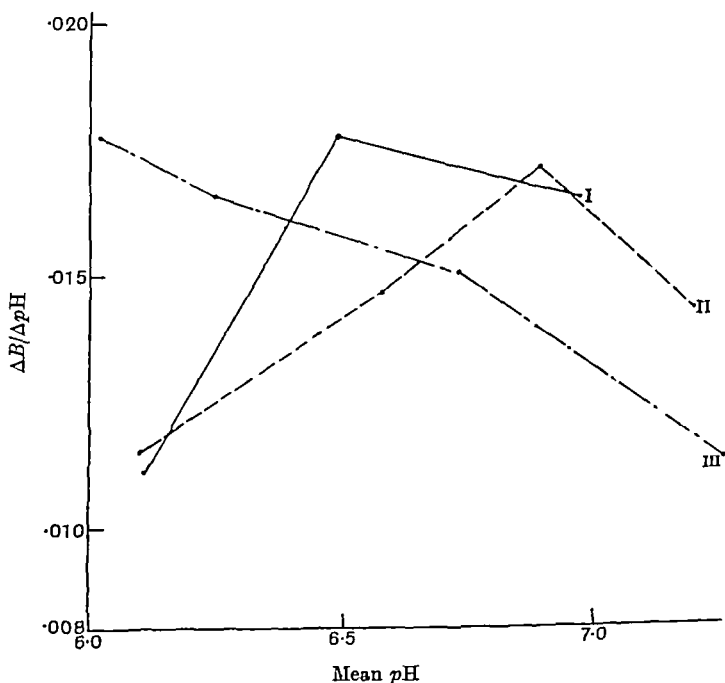


Fig. 8.

I=B before incubation. II=B' after incubation. III=C' after incubation.

TABLE XX. Buffering values. (Fig. 8.)

(B)				(B')			
B	pH	$\Delta B/\Delta pH$	Mean pH	B	pH	$\Delta B/\Delta pH$	Mean pH
—	5.89	—	—	—	5.80	—	—
0.005	6.34	0.0111	6.11	0.0075	6.40	0.0125	6.10
0.010	6.62	0.0178	6.48	0.0125	6.74	0.0147	6.57
0.020	7.22	0.0166	6.92	0.0175	7.03	0.0172	6.88
				0.0225	7.38	0.0143	7.20

TABLE XX (contd.).

(C')	B	pH	$\Delta B/\Delta pH$	Mean pH
	0	5.95	—	—
	0.0025	6.09	0.0178	6.02
	0.0075	6.39	0.0166	6.24
	0.0175	7.05	0.0151	6.72
	0.0225	7.49	0.0113	7.27

In the case of (B) and (B') the variations in the B.V. are not due to differences in the phosphate values as determined by chemical analysis. In (C'), on the other hand, the variations in the B.V., which are of the same type as have been previously described, are due to the distribution of the phosphate equally between the free and the bound forms. The maximal buffering in all three mixtures reaches in some reaction zone approximately the same value. The additive effect of two acids with dissociation constants of approximately  $pK'$  6.87 and 6.11 at the mean pH 6.02 (C') and acting in the concentrations determined by chemical analysis would give a B.V. about 0.005 less than that given (0.0178), so that substances other than the phosphates can secure base in this zone of reaction. As will be seen from experiments which will immediately be described when attention is directed to a zone of reaction not so far to the acid side (pH 6.3 to 7.5), the buffering of incubated mixtures, in which esterification has been very good both in the presence and in the absence of fluoride, indicates the presence of esters with a lower dissociation constant than  $pK_2'$  6.11.

At the mean pH 7.27 in (C') the weaker acid is the main buffer and the combined action of this acid and the stronger one in the concentrations existing would practically cover the B.V. determined, viz. 0.0113. The protein is acting as a buffer mainly on the acid side and less on the alkaline. If the two acids were acting in equal concentrations (as in this case they practically are) and no other buffer were present, then in the reaction zone examined (pH 6 to 7.2) owing to the overlapping of the buffer effects the B.V. values ought to be approximately the same throughout.

In order to determine approximately the strengths of the acids produced during the enzymic changes, one may compare throughout an electrometric titration the amounts of acid added to produce the various shifts in reaction with the difference in the base theoretically held by the phosphate systems before and after the addition of the acid, on the supposition that the free phosphoric acid has a  $K_2'$  in the KCl mixtures of  $1.7 \cdot 10^{-7}$  and the phosphoric acid ester one of  $10^{-6}$ . The difference in the base held between two pH values can then be stated in terms of the

acid used for titration and one can thus compare acid (or base) added with acid (or base) calculated. If these values do not correspond, then either the  $K_2'$  of the phosphoric acid ester is incorrect or some other buffering substance is parting with or securing base between the points examined and thus contributing to the buffering.

Two examples will be given to illustrate the procedure, and these are selected because in each case esterification was very well marked, in one case with lactic acid production, in the other, a fluoride mixture, without lactic acid production.

An unincubated mixture was titrated (1) before and (2) after deproteinisation by steaming, both being diluted to the same extent. The composition of the mixtures was as follows:

Free phosphate .....	0.0274 m.
Bound phosphate.....	0.001 m.
Lactic acid .....	0.0059 m.
Glycogen .....	8 mg. per c.c.
Reaction .....	7.31

To explain how the figures in the third column (Table XXI) were arrived at, the last figure in the column may be taken.

At pH 7.31 the base held by the free phosphate was 48.8 millimols, by the bound 1.9 millimols and by the lactic acid 5.9 millimols or 56.6 per litre in all. On adding 0.6 c.c. M./5 acid to 5 c.c. of the mixture the reaction shifted to pH 6.08, and at this reaction the base held by the

TABLE XXI. Electrometric titration.

The unincubated mixture.			The same deproteinised.		
pH	Volume of M./5 HCl added	Volume of M./5 HCl	pH	Volume of M./5 HCl added	Volume of M./5 HCl
	to 5 c.c. (c.c.)	calculated (c.c.)		to 5 c.c. (c.c.)	calculated (c.c.)
7.31	0	0	7.27	0	0
7.14	0.1	0.055	6.98	0.10	0.10
6.87	0.2	0.15	6.64	0.26	0.24
6.65	0.3	0.24	6.21	0.40	0.38
6.08	0.6	0.43			

three systems should have been 39.5 millimols per litre. Therefore the base calculated as removed by hydrochloric acid was 17.1 millimols, equivalent to 85.5 c.c. M./5 acid per litre or 0.43 c.c. M./5 HCl per 5 c.c. mixture. The electrometric titrations in this and in the following experiment were carried out, both in the unincubated and in the incubated mixtures, within a comparatively narrow zone of reaction around neutrality, and thus differed from some of the earlier series.

In this unincubated mixture the buffering was completely accounted

for by the protein and the phosphate systems present. The base held by the very small amount of ester present only ranged from 1.9 millimols at pH 7.32 to 1.5 at pH 6.08 and so the buffering in the original mixture due to the bound phosphate was negligible. The buffering of the deproteinised mixture was practically entirely due to the inorganic phosphate.

The mixture was incubated for  $4\frac{1}{2}$  hours at  $25^{\circ}$  and on analysis was found to contain after dilution to the same extent as a deproteinised portion:

Free phosphate .....	0.0127 m.
Bound phosphate.....	0.0157 m.
Lactic acid .....	0.0183 m.
Reaction (pH) .....	6.301

TABLE XXII.

The incubated mixture.			The same deproteinised.		
pH	M./5 KOH added to 5 c.c. (c.c.)	M./5 KOH calculated (c.c.)	pH	M./5 KOH added to 5 c.c. (c.c.)	M./5 KOH calculated (c.c.)
6.30	0	0	6.32	0	0
6.52	0.10	0.07	6.50	0.10	0.085
6.76	0.20	0.145	6.90	0.20	0.18
7.21	0.30	0.255	7.23	0.30	0.25
7.73	0.41	0.33			

After incubation the buffering of the mixture was better than that calculated for the two phosphate systems. This extra buffering might be due either to the formation of another substance, not present in appreciable quantity, in the original mixture and not destroyed by steaming or to the fact that the ester is a weaker acid than one with a  $K_2' = 10^{-6}$ .

The following considerations go to show that the latter of these alternatives is the more probable. The total base taken up by the inorganic phosphate, organic phosphate and lactic acid at the reaction previously mentioned for the respective mixtures should have been in

- (1) The original mixture 56.6 millimols.
- (2) The original mixture deproteinised 56.1 millimols.
- (3) The incubated mixture 60.4 millimols.
- (4) The same deproteinised 60.8 millimols.

If another substance were formed during incubation which would account for the extra buffering the base taken up by the lactic acid would bring the base content to a lower value than 56 millimols. instead of to the higher one. If the  $K_2'$  of the ester were taken as  $3 \cdot 10^{-7}$  instead of the higher value given by Meyerhof for the mono-ester. the total base in the incubated mixture would be approximately 55.8 and in the same mixture deproteinised 56.5.



Calculations based on this value for the ester gave results in better agreement with the experimental data.

Taking the deproteinised incubated mixture, the following represent the values:

Vol. M./5 KOH added to 5 c.c. (c.c.)	Vol. M./5 KOH calculated for an ester $K_2'$ $3 \cdot 10^{-7}$ (c.c.)	Vol. of M./5 KOH calculated for $K_2'$ $10^{-6}$ (c.c.)
0.10	0.09	0.085
0.20	0.22	0.18
0.30	0.32	0.25

The other example is chosen because the incubated mixture contained by far the greater part of the phosphate in ester form and owing to the presence of fluoride there was no evidence of lactic acid production. A muscle extract, with added phosphate and glycogen, similar to the preceding one was incubated for  $4\frac{1}{2}$  hours at  $25^\circ$  in the presence of fluoride. The unincubated mixture was not electrometrically titrated as the main buffering substances were simply the free phosphate and the protein. The incubated mixture before and after deproteinisation was electrometrically titrated. The composition of the mixtures was:

Free phosphate .....	0.0041 m.
Bound phosphate.....	0.0193 m.
Lactic acid .....	0.005 m.
Reaction .....	pH 6.52 (6.55 deproteinised).

TABLE XXIII. Electrometric titration.

Incubated fluoride mixture.		Same deproteinised.	
Vol. M./5 KOH added to 4 c.c. (c.c.)	pH	Vol. M./5 KOH added to 4 c.c. (c.c.)	pH
0.0	6.52	0.0	6.55
0.10	6.26	0.05	6.74
0.15	7.09	0.10	6.94
0.20	7.61	0.16	7.13
		0.20	7.84

Using the  $K_2'$  of  $H_3PO_4$  in fluoride solution as determined by Meyerhof  $1.82 \cdot 10^{-7}$  and  $K_2'$  (ester)  $10^{-6}$  the following results were obtained by the method already described.

TABLE XXIV.

Incubated mixture.			Same deproteinised.		
pH	Vol. M./5 KOH added to 4 c.c. (c.c.)	Vol. M./5 KOH calculated (c.c.)	pH	Vol. M./5 KOH added to 4 c.c. (c.c.)	Vol. M./5 KOH calculated (c.c.)
6.96	0.10	0.071	6.74	0.05	0.036
7.09	0.15	0.085	6.94	0.10	0.064
7.61	0.20	0.120	7.31	0.15	0.084
			7.84	0.20	0.123

Obviously the phosphoric acid ester is a weaker acid than one with a constant  $10^{-6}$ .

Taking  $K_2'$  for this acid as  $3.4 \cdot 10^{-7}$ , better agreement is obtained thus:

Deproteinised incubated mixture.

(1)	(2) (c.c.)	(3) (c.c.)
6.74	0.05	0.052
6.94	0.10	0.096
7.31	0.15	0.133
7.84	0.20	0.203

We would suggest, therefore, that in certain cases at least, such as those which have just been described, the buffering of the unincubated mixtures is mainly due to the inorganic phosphate and slightly to the small amount of protein present, while in the incubated mixtures the main buffering is accomplished by the mixture of the free and the bound phosphates and that the phosphoric acid ester at certain stages in the incubation process is an acid with a lower dissociation constant than  $10^{-6}$ . There is, however, a difficulty on this hypothesis in the explanation of the degree of acid swing which occurs as a result of the enzymic changes in fluoride mixtures. Taking the last example described, in the original mixture before incubation the reaction was 7.38 and the total base held by the free phosphoric acid and lactic acid equalled 47.7 millimols per litre. After incubation with  $pH$  6.53, taking  $K_2'$  (ester) as  $10^{-6}$ , the total base equalled 43.54 and for  $K_2'$  (ester)  $3.4 \cdot 10^{-7}$ , 41.22 millimols, held by the free  $H_3PO_4$ , the ester and lactic acid, but the electrometric titration indicated the presence of an ester with the lower constant and therefore it appears as if an acid had been produced with a sufficiently high dissociation constant not to add to the buffering capacity of the mixture in the reaction zone examined (6.5-7.5). This might simply be due to a slight increment in lactic acid which in the method of determination (Clausen) was not detected in the fluoride mixtures (*vide* Lipmann(10)) or to the fact that some other acid appeared which would naturally have withdrawn base during the incubation process and yet might not have added to the buffering capacity in the zone around neutrality.

One must remember, however, that the B.V. have been determined in incubated mixtures containing other constituents in small quantity which might contribute to the buffering in the zone of reaction examined. Hence a difficulty arises in assigning a particular dissociation constant value to the ester or esters produced.

## SUMMARY.

1. The enzymic changes associated with the breakdown of glycogen—esterification and glycolysis—occur when the expressed juice of the muscle, or saline extracts of the fibres from which the juice has been removed, are mixed with an alkaline phosphate solution containing glycogen.

2. When saline or similar extracts of muscle prepared by Meyerhof's method are incubated in bicarbonate solution at temperatures from 22° to 45° there is an approximate equimolecular production of phosphoric and lactic acids from the breakdown of the hexose diphosphate present in such extracts.

3. When glycogen alone is added esterification and lactic acid production occur in approximately equimolecular proportion in mixtures incubated at 22°. During this process the inorganic phosphate may entirely disappear. At 37° both esterification and lactic acid production are diminished, but the equimolecular proportions are still maintained. At 45° esterification ceases but the diastatic action and the breakdown of the pre-existing hexose diphosphate still proceed.

4. When additional phosphate is added to the glycogen-holding mixtures, esterification becomes the dominant process at 22°. At 37° esterification is less and lactic acid production greater than at the lower temperature. At 45° there is still some esterification along with lactic acid production, but at 50° both processes are inhibited although the diastatic action is still evident.

5. In the presence of fluoride esterification alone occurs and is always greater than in its absence.

6. The time course of the reaction and of the chemical changes was studied in a series of mixtures incubated at different temperatures. The characteristic rise in acidity is the accompaniment of the esterification process with or without lactic acid production.

7. Esterification of itself, owing to the formation of esters with higher dissociation constants than orthophosphoric acid, results in a fall in the buffering capacity in the neighbourhood of the neutral point and a rise in the more acid zone from pH 6.5 to 5.5.

Various unincubated and incubated mixtures, with and without protein, were electrometrically titrated and their approximate B.V. determined in different zones of reaction. When the zone around neutrality (pH 6.5 to 7.5) is examined, it appears that at least in some

cases the esters which are produced are acids with a lower dissociation-constant value than  $pK' 6$  or  $6.11$ .

We are much indebted to J. C. Davison and J. H. Strahan for assistance in carrying out analyses.

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# THE INFLUENCE OF MECHANICAL FACTORS OF THE CIRCULATION UPON THE HEART VOLUME.

By E. PESERICO (*Rockefeller Travelling Fellow*).

(*From the Physiological Laboratory, Cambridge.*)

STARLING and his co-workers (1, 2, 3) showed that any increase in the output of the heart, or in the resistance against which it contracts, is accompanied by an increase in the heart volume. Investigations upon the oxygen consumption of the heart led to a further important observation. It was found that the oxygen consumption is determined, under a constant physiological condition of the heart muscle, by its diastolic volume (4, 5, 6) and that, in the case of weakening of the heart, although the work is maintained constant, the diastolic volume and, therefore, the oxygen consumption increase, the work being performed with a smaller efficiency. We have, therefore, on the one hand a dependence of oxygen utilisation on the heart volume and, on the other hand, a dependence of the heart volume (under constant conditions) on circulatory factors such as output and arterial pressure. Investigations of the relation of the heart volume to the resistance and output are few and none have been made with any degree of quantitative precision.

Lovatt Evans and Matsuoka (4) observed that an increase in work produced by changing the arterial pressure leads to a greater increase in the oxygen consumption than an equal increase in work produced by changing the output. Since, according to his experiments, the oxygen consumption is a function of the heart volume Lovatt Evans predicted that an increase in arterial pressure would lead to a greater dilation of the heart than an equal increase in output. This was not, however, supported by direct observations; moreover Lovatt Evans' calculation of the work done by the heart is open to criticism on several points<sup>1</sup>. Dr R. Kinoshita found in a series of unpublished experiments with the tortoise heart that within a certain range changes of output and of pressure have an equal effect upon the heart volume or, in other words, that within this

<sup>1</sup> In Lovatt Evans' experiments the pulmonary blood-pressure was not measured but taken as  $\frac{1}{2}$  of the aortic pressure which, depending on the output of the heart, may introduce an error of over 100 p.c. in the calculation of the work done by the right ventricle. The coronary blood flow was in most experiments calculated on a constant basis of 0.6 c.c. per gram. of heart per minute.

range the changes in the heart volume are proportional to the product of output and pressure, *i.e.*, to the external work. Starling and Visscher(5) working on the mammalian heart confirmed these observations, pointing out, however, that the relation between the external work and the heart volume holds true only for a certain number of cases. At the suggestion of Dr G. V. Anrep I undertook to reinvestigate the comparative effect of changes of pressure, output and heart rate on the heart volume.

*Method.* The experiments were performed on the isolated tortoise ventricles in which it is easy to control the experimental conditions and also to calculate the work performed and to measure the cardiac volume. The apparatus used was modelled after that of Kozawa(7) except that a sensitive membrane manometer (vibration frequency 25-30) was connected with the outlet tube of the cannula so as to measure the intraventricular pressure. The rate of the isolated ventricle was controlled at 15 beats per minute; oxygenated Ringer's fluid of pH 7.6 was used for perfusion. In calculating the work done by the ventricle the kinetic factor was disregarded which at large outputs introduced an error not exceeding 1.5 p.c. With the usual outputs this error was considerably smaller. The ventricular volume was measured with an accuracy of 0.01 c.c. The arterial resistance was arranged so as to allow only a minimal change in pressure (not more than 0.1 cm. of  $H_2O$ ) during systole; these changes were neglected in the calculation of the work.

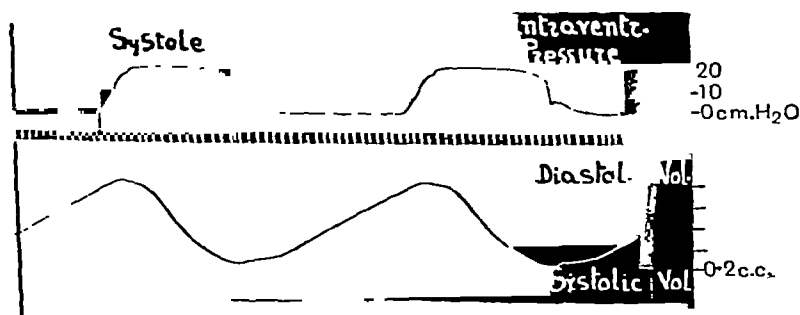


Fig. 1. Volume and pressure changes of the isolated ventricle. From above downwards; intraventricular pressure, time in 0.1 sec., volume changes of the beating ventricle and volume of the empty heart.

The perfusion cannula was introduced into the ventricle through the auricle and was tied around the *a.-v.* groove; all the vessels coming from

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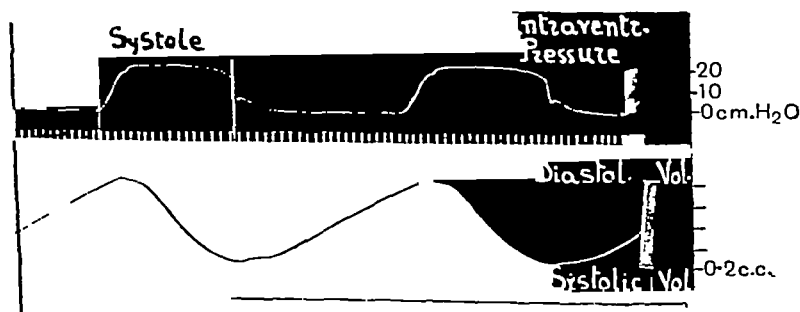


Fig. 1. Volume and pressure changes of the isolated ventricle. From above downwards; intraventricular pressure, time in 0.1 sec., volume changes of the beating ventricle and volume of the empty heart.

The perfusion cannula was introduced into the ventricle through the auricle and was tied around the *a.-v.* groove; all the vessels coming from



the heart were ligatured separately. The cannula which was made of silver consisted of two tubes one of which was connected with the venous reservoir, the other with the arterial resistance, sensitive valves being placed on both sides of the heart. One stimulating electrode was placed on the silver cannula the other on the outside of the heart close to the ligature. Fig. 1 shows a tracing of the heart volume and of the intra-ventricular pressures as obtained in these experiments.

*Effect of changes in output and pressure on the relation between work and the ventricular volume.* The relation between work and volume ( $w/v$  ratio) can be taken to represent the physiological condition of the cardiac muscle. A heart which contracts strongly performs a given work with a smaller volume and on increasing the work dilates less than a heart the contractility of which is impaired. The importance of this ratio is obvious since it ultimately determines the limits of cardiac activity.

In some of the experiments described below the arterial pressure was maintained constant while the output was changed, in others the output was kept constant while the pressure was changed. Fig. 2, in

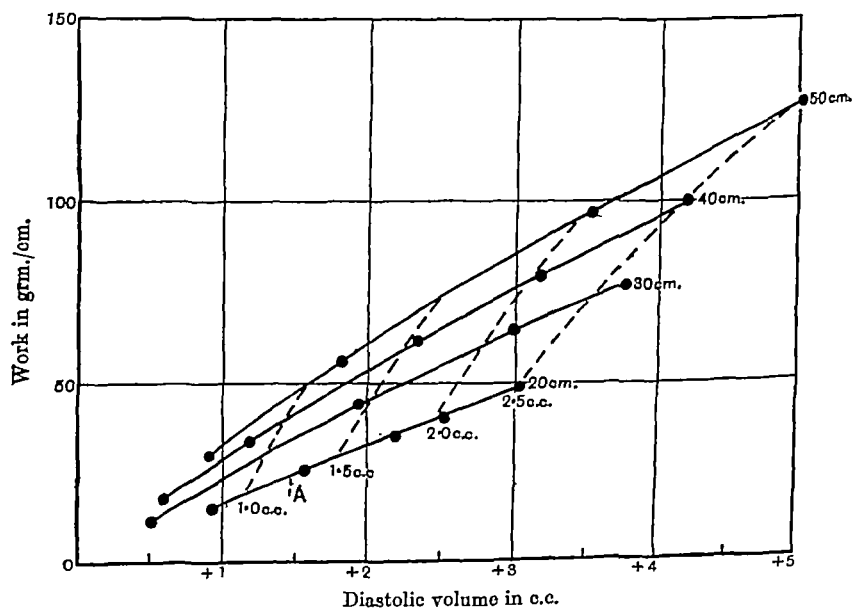


Fig. 2. Work-volume diagram. Continuous lines—effect of changes in work produced by varying the output at constant pressures (20, 30, 40 and 50 cm. H<sub>2</sub>O). Interrupted lines—effect of changes in pressure at constant output (of 1.0, 1.5, 2.0 and 2.5 c.c.). Abscissa—increase of the ventricles volume above the volume of the empty ventricle which in this experiment was equal to 2 c.c.



The difference in the effect of resistance and output becomes smaller at higher pressures, which can be seen from Fig. 3, taken from an experiment in which the diastolic volume of the heart was kept constant at

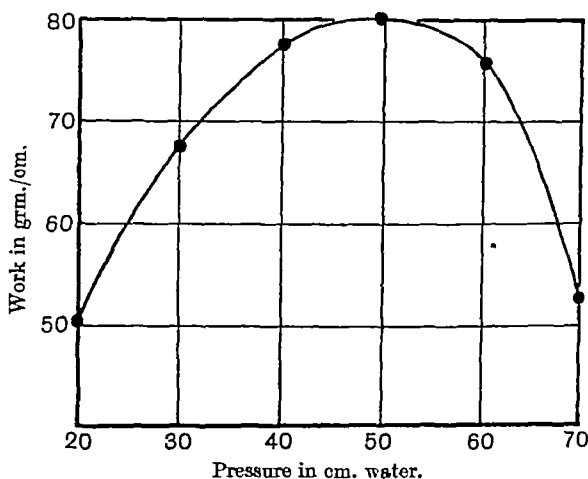


Fig. 3. Effect of pressure upon the work performed by the heart at constant diastolic volume. The diastolic volume is kept at 3 c.c. above the volume of the empty heart.

3 c.c. above the volume of the empty heart. The pressure was in this experiment gradually increased while the output was diminished so as to keep the diastolic volume constant. With a pressure of 30 cm. the work was 35 p.c. greater than with a pressure of 20 cm.; whereas between the pressures of 40 and 50 cm. the difference was negligible. At the higher pressures the diastolic volume may seem at first to be influenced to an equal extent by the output and pressure. However, more careful measurements disclose that here also a change in the output has a bigger effect than an equivalent change in the pressure. The  $w/v$  ratio increases only to a certain maximum after which it gradually declines, finally falling to zero when the point of isometric contraction is reached. The fall of the  $w/v$  ratio at very high pressures is the first sign of failure of the heart, the pressure has now a considerably greater effect upon the heart volume than an equivalent change in output. The maximum  $w/v$  ratio at which the work is performed with a relatively minimal volume as well as the slope of the work-volume diagram (Fig. 2) is typical for any one heart and for any condition of the heart. The  $w/v$  ratio is high in all cases when the heart is in good condition. An impairment in the contractility of the heart produces the following changes in this ratio. Under constant output and pressure the  $w/v$  ratio declines the work

being now performed with a larger diastolic volume. Furthermore the increase in the pressure leads in the weakened heart to a much smaller rise of the  $w/v$  ratio than in a normal heart. Under these conditions there may be a considerable range of pressure changes in which the changes in the ratio are so small that the work performed at a constant diastolic volume will be approximately the same at different pressures, i.e., that the pressure and output will have an equal effect upon the volume. Improvement in the condition of the heart leads to an immediate rise in the  $w/v$  ratio and to an accentuation of the effect of pressure upon the ratio. An example of such an effect is shown in Table II in

TABLE II. Action of adrenaline upon the value of  $w/v$ .

Arterial pressure in cm. H <sub>2</sub> O	Value of $w/v$	
	Before adrenaline	After adrenaline
10	5.9	9.1
20	7.1	17.5
30	5.2	25.0
40	—	31.0
50	—	34.0
60	—	30.5

which the contraction of the heart was strengthened by administration of adrenaline. It can be seen that adrenaline brought about an increase in the  $w/v$  ratios for all the pressures, that it accentuated the difference between the values of the ratio at different pressures and that it raised the optimal resistance at which the work is performed with the relatively smallest volume to a higher level.

Before administration of adrenaline the ventricle could not overcome pressures higher than 30 cm. while the highest  $w/v$  ratio is observed at a pressure of 20 cm. After adrenaline (concentration about 1 : 1,500,000) the  $w/v$  ratios are raised throughout and the highest ratio is at a pressure of 50 c.c.

It is tempting to compare the results of these experiments with the result of Starling's experiments upon the oxygen consumption of the mammalian heart. If the oxygen consumption is determined by the diastolic volume and is independent of the form of the work (whether it is mainly to overcome resistance or perform an output) then the  $w/v$  ratio must increase and decrease with the mechanical efficiency of the heart and it should be concluded from all the above experiments that the efficiency of the tortoise heart rises steeply with increase in pressure and diminishes slightly with increase in output.

*Effect of changes in output and pressure upon the length of the systole*

*and rate of contraction.* The experiments of Starling<sup>(3)</sup> and of Wiggers<sup>(8)</sup> show that at a constant arterial pressure an increase in the output invariably lengthens the duration of the systole. As regards the effect of changes in pressure opinion is divided. Patterson, Piper and Starling<sup>(2)</sup> found that the systole lengthens with rise in pressure while Wiggers finds that it shortens. So far as the tortoise ventricle is concerned our experiments support those of Wiggers. Increasing the output at a constant pressure prolongs the systole, while increasing the pressure at a constant output shortens it (Table III *a*). However, beyond

TABLE III.

(*a*) Data from a typical experiment showing the effect of increase in pressure on the duration of the systole. Output per beat 0.75 c.c., heart rate—15 per minute.

(*b*) Same as in *a* but with the heart in rather poor condition. At a pressure of 40 cm. the systole begins to lengthen and the value of the  $w/v$  ratio begins to decline. Output per beat 0.85 c.c., heart rate—15 per minute.

The figures in *italics* show the simultaneous prolongation of systole and fall in  $w/v$  ratio.

<i>a</i>			<i>b</i>		
Pressure in cm. H <sub>2</sub> O	Length of systole in sec.	$w/v$	Pressure in cm. H <sub>2</sub> O	Length of systole in sec.	$w/v$
10	2.17	10	10	2.03	6.3
20	1.93	19	20	1.95	11.3
30	1.75	26	30	1.85	14.3
40	1.63	35	40	1.87	14.0
50	1.55	42	50	1.96	10.4
40	1.67	36	40	1.89	12.9
30	1.75	27	30	1.84	13.8
20	1.83	19	20	1.87	10.2
10	2.10	10	10	2.00	5.7

a certain pressure the systole again slightly lengthens and this, confirming Wiggers, is often accompanied by an increase in the initial pressure (measured with an accuracy of 0.5 cm. H<sub>2</sub>O). It is an interesting fact that the fall in the  $w/v$  ratio in the region beyond the optimal pressure and the prolongation of the systole begin roughly at the same arterial pressure (Table III *b*). In a weak heart adrenaline besides raising the height of pressure at which the  $w/v$  ratio begins to fall also produces a similar shift of the point at which the systole begins to lengthen.

In the light of A. V. Hill's work the fact that an increase in the output prolongs the systole while an increase in the pressure shortens it would lead one to expect a rise in the  $w/v$  ratio in the first case and a decline in the second, the same work being performed in a shorter time at higher pressures. However, a change in the duration of the systole does not necessarily mean a corresponding change in the rate of contraction of the heart and this for the following reasons.

In the tortoise ventricle the systole may outlast the duration of the ejection phase sometimes for a considerable time, the contraction of the ventricle persisting in an isometric form after all the work has been done. This outlasting of the contraction can be observed with almost any possible output provided the arterial pressure is low. A rise in pressure leads to a more uniform distribution of the work over the whole length of systole so that less energy is lost in the ineffective maintenance of contraction and the work is performed at a more uniform and therefore at a slower rate. This difference in the response is well illustrated in the redrawn and superimposed tracings of Fig. 4. In this experiment the

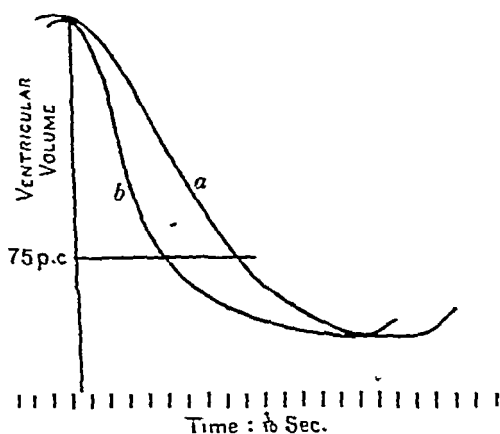


Fig. 4. Redrawn superimposed tracing of the ventricular volume during one systole; *a*—at an arterial pressure of 50; *b*—at a pressure of 20 cm.  $H_2O$ . The line drawn across the volume curves intersects the points at which 75 p.c. of the work was completed.

output per beat was 1.3 c.c. In the case of the lower pressure 75 p.c. of the work was performed in 0.5 sec. while in the case of higher pressure it was performed in 0.9 sec. It is, therefore, obvious that in spite of some shortening of the systole the maximum rate of contraction became considerably slower at the higher pressure. This effect of pressure is not equally conspicuous in every heart. There is, however, another consideration which also serves to show that during constant work the rate of contraction is considerably faster when the main component of the work is the performance of an output and somewhat slower when it is to overcome a resistance and that this is in spite of lengthening of the systole in the first case and shortening in the second. The difference in the circumferences of two spheres whose volumes are respectively the diastolic and the systolic volumes of the ventricle for a given output can

be taken as a rough measure of the shortening of the ventricular fibres. Taking now for instance from Table III *b* the figures for pressures 10 and 40, and knowing that the volume of the empty heart was 2.2 c.c., it is easy to calculate<sup>1</sup> that at pressure 40 the shortening of the fibres corresponds approximately to 3.9 mm., and as it is performed in 1.67 sec. its average rate is 2.3 mm. per second. At the pressure 10 the shortening corresponds to 5.1 mm., and is accomplished in 1.87 sec. at an average rate of 2.75 mm. In experiment III *b* the heart was in a rather poor condition and it is obvious that the weaker the heart the bigger is the dilation produced by a given increase in pressure and, therefore, the greater is the slowing of the mean rate of contraction. In a heart which is in a good condition the slowing of the calculated rate of contraction may be nearly completely compensated by the slight shortening of the systole. On the other hand increasing the output at a constant pressure always increases the rate of contraction in spite of prolongation of the systole and of the dilation of the heart. Fig. 5 shows the extent to which the rate of contraction as calculated for the experiment given in Fig. 1 is affected by changes in the resistance and in the output. On account of these changes in the rate of contraction it is to be expected that for a given increase in work the heart should dilate more when the increase is brought about by changing the output than when it is brought about by changing the pressure. In the first case the heart besides performing additional work has to do it with a faster rate of contraction while in the second case the rate of contraction is slightly diminished. It is obvious that these calculations cannot be accepted without considerable reservation since the fibres of the heart are not circular, nevertheless the course of fibres is constant for any heart and, therefore, the average rate of contraction as calculated here should be proportional to the actual average rate of contraction.

All the above experiments were performed with the isolated ventricle the beat of which was controlled by electric stimulation. This is an important condition since the spontaneous rhythm of the ventricle is usually not quite constant, moreover it changes when the resistance or the output are increased, decreasing in the first case and increasing in the second. Even small changes in heart rate affect the diastolic volume, therefore the work, the duration of systole and the rate of contraction change from beat to beat.

<sup>1</sup> The rate of contraction was calculated as  $\frac{3.9(\sqrt[3]{v} - \sqrt[3]{v_1})}{T}$  where  $v$  is the diastolic volume,  $v_1$  the systolic volume and  $T$  the duration of the ejection phase.

Every change in heart rate is immediately reflected upon the  $w/v$  ratio. The tortoise ventricle assumes a bigger volume when it has to

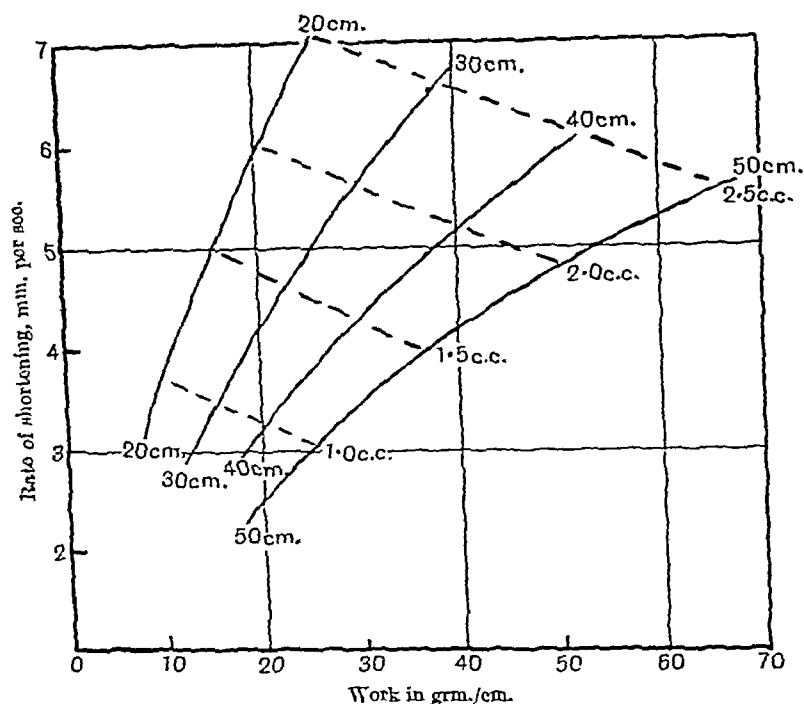


Fig. 5. Effect of change in work upon the rate of contraction by varying output at constant pressures of 20, 30, 40 and 50 cm.  $H_2O$  (continuous lines) and by varying pressures at constant outputs of 1.0, 1.5, 2.0 and 2.5 c.c. per beat (interrupted lines). The rate of contraction is calculated as described in the text. The data for this diagram were taken from Table I.

perform a constant amount of work per beat at faster rate per minute; consequently the  $w/v$  ratio declines whenever the heart is accelerated. In other words if the diastolic volume is kept constant the heart is able to perform less work when it beats faster. The extent of this effect can be seen from Table IV.

TABLE IV.

Arterial pressure in cm. $H_2O$	Work per beat in gm./cm.	Diastolic volume	$w/v$	Heart rate
20	31.2	1.92	17.8	15
	27.0	1.92	14.0	20
30	43.5	1.71	25.5	15
	34.2	1.71	20.0	20
40	97.2	2.70	36.0	15
	82.8	2.70	30.5	20



The diminution in the  $w/v$  ratio at faster heart rates conforms well with the observations of Starling and Visscher who show that the heart muscle similarly to other contractile tissues is less efficient at faster rates.

#### CONCLUSIONS.

1. The effects of changes of output, arterial resistance and rate upon the relationship between work and diastolic volume, and upon the duration of the systolic response of the isolated tortoise ventricle have been studied.

2. At constant resistance and constant rate every increase in output is followed by a slight diminution of the value of the ratio between work per beat in grm./cm., and diastolic increase in volume over the volume of the empty ventricle. The larger the output the less the amount of work which corresponds to every unit increase of the diastolic volume.

3. If output and rate are kept constant and the arterial resistance is raised, the value of  $w/v$  increases and reaches a maximum at an optimum resistance, beyond which it decreases. The position of the maximum varies from heart to heart and with the condition of the heart.

4. When the rate is increased the ventricle dilates to a larger diastolic volume in order to perform the same work per beat as before and consequently the value of  $w/v$  decreases.

I wish to express my thanks to Dr G. V. Anrep for his help and criticism during my experiments. The expenses of this research were defrayed by grants (a) from the Rockefeller Foundation to the author and (b) from the Medical Research Council to Dr Anrep.

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## EXPERIMENTS ON VISCERAL SENSATION.

Part II. The sensation of "nausea" and "sinking";  
œsophageal reflexes and counter-irritation.

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IN Part I<sup>(1)</sup> it was shown that œsophageal pain results from a stretching force in the viscus, and the theory was put forward that it is due to tension exerted on pain nerve endings in the wall. Pain is relieved by a peristaltic contraction which overcomes the stretch, or by postural adaptation which increases the capacity of the viscus, so that the stretching force—in most cases a rubber bag containing air—becomes less. Tonus was defined as the tension of the plain muscle during diastole. Pain produced experimentally is associated with high tonus.

The method of experiment in Part II was that already described in Part I, and we ourselves were the subjects of nearly all the experiments. The capacity of all bags was 50 c.c. unless special mention is made. When filled inside the œsophagus the shape of the bag was cylindrical (see Part I, Fig. 7) and stretching of the œsophageal wall took place for a length of about  $2\frac{1}{2}$  in. above the lower end of the bag. Since the diameter of the œsophagus varied but slightly in these experiments the tonus was measured by the diastolic pressure.

### *The sensation of nausea.*

On questioning people there is evidently disagreement as to what sensation the term "nausea" or "feeling of sickness" is commonly applied. In the present communication the term is defined as a sensation which is felt at the back and lower part of the throat; it is not usually associated with pain. At the same time we admit that this sensation is often accompanied by uneasiness in the pit of the stomach, which is denoted by the term "sinking sensation"; while the brow-ache which is

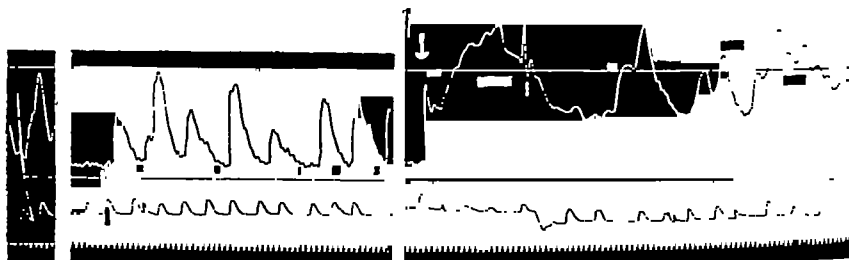
<sup>1</sup> Part of the work was carried out during the tenure by W. W. Payne of the Parsons Research Fellowship at Guy's Hospital and by E. P. Poulton of a Beit Memorial Fellowship. The expenses were defrayed by means of a grant from the Government Grants Committee of the Royal Society.

also not an uncommon accompaniment of these sensations must be produced reflexly, and has not been investigated.

*Exp. 1.* (Fig. 1 A and B.) May 25th, 1922. Subject E.P.P. In this subject it was found by X-ray that the cardia and xiphisternum were on the same level. The cardia was  $19\frac{1}{2}$  in.

A

B



C

D

Fig. 1 A, B. *Exp. 1.* Oesophageal tracings. Pressure shown in cm. water. Lower horizontal line 0 cm.; upper 40 cm.; middle line shown in B 20 cm. Lower tracing, respiration; inspiration is an upstroke on the respiratory tracing and a down stroke on the oesophageal tracing. Time in seconds. Simultaneous points on two tracings are indicated by short vertical lines. Other figures, arranged similarly. A, Bag contained 30 c.c. air. White rectangles indicate nausea of varying duration. B, Bag 40 c.c. Rectangles above tracing represent nausea, and rectangles below show where pain was signalled. Their vertical projection on to the tracing gives the time relation.

Fig. 1 C and D. *Exp. 2.* Tracing from the cardiac part of the stomach. Horizontal lines 0, 20 and 40 cm. White rectangles indicate sensation of sinking. An upstroke shows inspiration. Between C and D 200 c.c. air introduced into the stomach.

and the oesophageal level corresponding to the suprasternal notch was  $9\frac{1}{2}$  in. below the teeth. A preliminary experiment (not shown in this figure) had been carried out during a period of 6 min. in which the bag, containing 40 c.c. air, situated 13 in. below the lips was eventually vomited. The bag was swallowed again and was kept with its lower end 12 in. from the lips. It contained 10 c.c. of air during a period of  $2\frac{1}{2}$  min. The diastolic pressure varied between 0 and 4 cm. Oesophageal contractions occurred every 4 sec., the systolic pressure being never higher than 30 cm. The volume was then increased to 30 c.c. for over 5 min. and the diastolic pressure rose to 6 or 7 cm., while the systolic pressure was now often up to 40. The activity was more pronounced than before, but during three separate periods, totalling 1 min., there were no contractions, and the diastolic pressure was 3–5 cm. During the later half of the time, shown at A, a momentary sensation of nausea was signalled repeatedly at the end of relaxation or while relaxation was taking place, and

thus the tracing resembled the records of pain shown in Figs. 4 and 5 of Part I. The air in the bag was then (at the arrow in *B*) increased to 40 c.c. and the diastolic and systolic pressures rose immediately. Nausea continued to be signalled nearly always during relaxation; but pain was felt as well. After about 70 sec. the element of nausea vanished from the sensation, and pain in the throat was alone experienced.

Measurement of *A* shows that nausea was signalled alone when the diastolic pressure was on the average 6 cm. In *B* nausea was signalled at 21 cm. immediately after the air in the bag had been increased to 40 c.c. and then pain in the throat at 43 cm. and subsequently nausea or pain were signalled. Actually the subject felt that there was a transition in the sensation from nausea through painful nausea to pain alone; so that the sensations recorded were really mixed, with nausea or pain predominating as the case might be. It proved rather difficult to determine exactly when the painful nausea ended, and the pure pain began. After 3 min. pain was felt behind the sternum, and at this point the pain in the throat disappeared. It cannot be said that there was any difference in the record to correspond with the change in the position of the sensation, and the position of the bag had not altered.

Several other experiments on nausea were carried out in the case of both of us with similar results. The fact that this sensation was felt during œsophageal relaxation suggests that the mechanism of its production is essentially the same as for visceral pain, but because the diastolic pressure is lower with nausea than with pain, a different type of end organ is probably involved, which reacts to a lower degree of tension. The relation of nausea and œsophageal pain is thus very similar to the sensations of pressure and painful pressure on a skeletal muscle, or of touch and pin prick on the skin. In each case a slight stimulus produces the specific sensation and the latter gradually disappears completely as the stimulus increases, and finally pain alone is left. Further, the intermediate stage of a painful nausea strongly suggests that the end organs for nausea and pain are different, and that both may be stimulated at the same time; but, as is invariably the case elsewhere, pain is the over-riding sensation when the stimulus increases. It is noteworthy that the nausea was felt in a rather higher position than the bag which caused it, but the higher the bag is placed in the œsophagus, the more readily is the sensation produced.

The sensation of nausea in these experiments was also described as a sensation of "sinking in the throat"; but if, as we have suggested, it is due to the effect of increased tension exerted on specific end organs other methods of increasing this tension would result in this same sensation. One of us E.P.P. has observed, while standing during a channel crossing,

that the sensation of nausea is felt during the descent of the ship, but vanishes during its ascent. Dr G. H. Oriel who has studied seasickness<sup>(2)</sup> has informed us, that it is during the retardation towards the bottom of the descent that the sensation is noticed. This would tend to cause a dropping of the unsupported soft parts and consequently an increased degree of tension in the specific end organs for nausea in the œsophageal wall.

### *The sensation of sinking.*

*Exp. 2.* (Fig. 1 c and d.) Jan. 5th, 1922. Subject W.W.P. A rubber catheter and rubber bag were passed into the cardiac part of the stomach. The catheter was connected with a bellows so as to be able to force air rapidly into the stomach. The bag contained 20 c.c. air; a fairly level tracing (c) was obtained; the diastolic pressure was 19–22 cm. The small variations on the tracing were respiratory as was proved later when a simultaneous stethographic tracing was also taken. 200 c.c. of air were then introduced into the stomach; the effect of this was to cause a slight periodic rise of the tracing to 26 cm. and a fall to 20 cm. (d). A feeling of sinking lasting about a second was recorded repeatedly during the descent but very near the lowest point of the tracing. Simultaneously a desire to eructate was also felt. The latter sensation is described later.

The small rises of pressure—only 6 cm.—were probably due to contractions in the pyloric part of the stomach. They were not merely rises in tonus because their periodicity was quite short, the intervals varying from 9 to 25 sec. with an average value of 16; also they corresponded with the œsophageal contractions described in the next section. They evidently constitute Carlson's 20 sec. rhythm<sup>(3)</sup>. Prof. Carlson in a personal communication has kindly stated that he regards this rhythm as due to peristalsis and not to changes in tonus.

But there is further evidence on this head. In a previous communication<sup>(4)</sup> using a small water bag we showed that quite high pressures were often obtained from the pyloric part of the stomach due to movements here; but that the corresponding variations in the air bubble at the cardiac end were sometimes much smaller (Cases 11 and 12). There are two possible reasons for this. In Case 1 who had the typical elongated stomach of visceroptosis we found that the walls sagged together separating the stomach into two compartments, and the pressure variations in the upper compartment bore no relation at all to those in the lower. Secondly in the more usual case where the two parts are connected it is still only the pyloric part that contracts. This forces up the fluid and so increases the hydrostatic pressure acting below without increasing to the same degree the pressure in the air bubble above.

The suggestion is, then, that the sensations of sinking felt in the abdomen were due to events taking place in the lower part of the stomach which were recorded indirectly by an air bag at the cardiac end. On the

analogy of what has been said as to the cause of nausea in the œsophagus it would be reasonable to suppose that the sensation of sinking was due to a slight increase in the tonus of the walls of the stomach sufficient to cause stretching of specific sensory end organs, but not enough to cause pain, and that the peristaltic wave by causing contraction of the muscle abolished the stretch, so that the sensation was only felt during the relaxation at the lowest point of diastole. However in Exp. 2 blowing up the stomach with 200 c.c. air caused no rise in diastolic pressure at all; but since an increase in the diameter of an organ causes a rise of tonus<sup>(1)</sup> although the pressure remains the same, we may still look on the sensation of sinking in Exp. 2 as due to a rise in tonus. In another experiment on W.W.P. (May 11th, 1922) a rise of diastolic pressure from 8 to 19 cm. and again to 22 cm. was measured on passing first 800 and later a further 600 c.c. air into the stomach; the pressure eventually settled down to 12 cm. and a similar series of waves were produced with a periodicity of 16–25 sec. The two sensations—sinking, and a desire to eructate—were again noticed between these waves. In an experiment on E.P.P. the periodicity of the gastric waves was rather longer while no sinking sensation was produced, only a desire to eructate, and a slight burning sensation was also sometimes felt at various levels behind the sternum.

The desire to eructate was felt deep in the mid line about the level of the clavicle. The feeling was that of a lump or “globus” situated here which would be dispersed if the eructation were carried out, but no eructation necessarily followed. The sensation might also be described as a feeling of “wind,” and it must clearly be analogous to the well known “globus hystericus.” We regard this sensation of “globus” as being different in quality from nausea though the two are obviously closely related, since they are felt in approximately the same part of the body, and occur during the relaxations of an œsophageal peristaltic contraction, if the tonus is slightly increased.

#### *Gastro-œsophageal reflexes.*

We have just described the feeling of “globus,” which was produced by passing air into the stomach. This type of gastro-œsophageal reflex was investigated more fully in the following experiment.

Exp. 3 (Fig. 2 A.) Jan. 5th, 1922. Subject W.W.P. A catheter was passed into the stomach and a bag was placed with its end about 15½ in. below the teeth. In W.W.P. the cardia is 17½ in. below the teeth. A level tracing was obtained showing respiratory waves and a rather larger wave which was probably a local contraction. At the arrow 600 c.c. air were introduced through the catheter into the stomach, as described in Exp. 2, which

had been carried out just previously. The result was to cause a series of large contractions to pass down the œsophagus due to secondary peristalsis and not to swallowing. The sensation of sinking and of globus were again felt and recorded near the bottom of the depressions between the contractions. Hence the result of blowing up the stomach was not only to cause contractions in this organ but reflexly to produce simultaneous contractions in the œsophagus, since in both experiments the sensations of sinking and of globus were recorded between the contractions, and near the lowest points of the tracings.

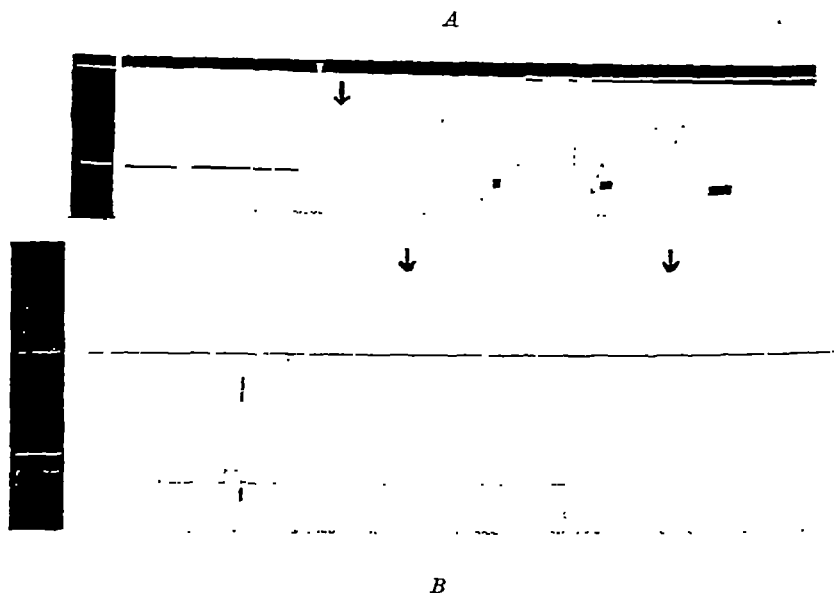


Fig. 2. Cœsophageal tracing. Horizontal lines 0 and 40 cm. *A*, Exp. 3. White rectangles indicate combined sensation of sinking and globus. *B*, Exp. 4. Upper signal—rise of lever shows retrosternal pain. Lower signal—epigastric pain.

We have already called this reflex the gastro-œsophageal anti-regurgitation reflex (4), but we have now found that it varies according to the condition of the œsophagus at the time it is elicited, as shown by the following experiment.

*Exp. 4.* (Fig. 2 *B*.) Jan. 12th, 1922. Subject W.W.P. Catheter and bag arranged as in Exp. 3. The lower end of the bag was  $14\frac{1}{2}$  in. below the teeth. At first 50 c.c. were placed in the bag; there was continuous burning retrosternal pain at a diastolic pressure of 31–35 cm. with complete absence of œsophageal contractions; a little air was then let out of the bag, and a series of contractions followed causing intermittent relief of pain. These are shown in the first half of the tracing. The diastolic pressure was 21 cm. 500 c.c. of air were forced into the stomach between the arrows on the tracing. This caused a complete cessation of œsophageal movements for a period of 16 sec.; then there was a contraction and another pause of 13 sec. and after this a mixed series of local and peristaltic contractions not all shown in the figures. The rhythm of the œsophageal activity had been completely upset by blowing up the stomach.

Previous to the blowing up intermittent œsophageal pain was felt during the relaxation of each of the large peristaltic waves shown in the tracing; but it was not recorded just previous to and at the beginning of the blowing up. The latter caused relief from the pain, but for only a few seconds. Then an additional pain was felt situated in the epigastrium due to distension of the stomach. This gradually passed off as the stomach readjusted its capacity to the increased amount of air which it now contained. The momentary relief from the œsophageal pain was not accompanied by any fall in diastolic pressure, so that it cannot have been due to relaxation of tension on pain nerve endings, which would be expected on our theory of visceral pain. It may have been due to the fact, that the attention was suddenly diverted from this pain to the other sensations produced by blowing up the stomach. It is a familiar observation that any painful sensation becomes momentarily diminished if some other occurrence suddenly distracts the attention.

*Various œsophageal reflexes.*

We did not in this piece of work set out to investigate all the possible ways of producing reflex activity in the œsophagus. What chiefly interested us were the cutaneo-œsophageal reflexes, owing to their relation to the therapeutic measure counter-irritation. However, it was necessary to investigate certain other reflexes since these might also be involved in counter-irritation experiments. A summary of 21 experiments on ourselves is given in this section.

Mosso and Pellacani(5) noticed that the bladder not only contracted spontaneously, but also when the patient was subjected to any emotional stimulus. Similar relations between emotion and the electrical resistance of the skin constitutes the well known psycho-galvanic reflex. This subject has been reviewed by McDowall and Wells who conclude that the fall of resistance resulting from emotion is due to vasoconstriction(6). We did not find that threatening the subject (W.W.P.) with a lighted match or suddenly flashing a bright light or making a loud noise produced any effect on the œsophageal activity as tested by means of a bag containing 10 c.c. On two occasions a noise was made during an apnoea following a period of forced breathing; there was also no effect. In all, seven observations were made.

On the other hand the smell of ammonia produced œsophageal activity both in the case of W.W.P. and E.P.P. (four expts.). This is of importance since many liniments contain ammonia or other pungent substances, and the possibility of a trigeminal visceral reflex must be



considered. This agrees with the well known observation that on smelling ammonia the heart is slowed by vagal action.

In two experiments swallowing ice-cold water abolished completely the activity of E.P.P.'s œsophagus which had been excited by the presence of a bag containing 40 c.c. of air. On the other hand when warm water was swallowed shortly afterwards no such effect was produced.

On several occasions we noticed that talking increased the activity of the œsophagus. On the other hand reading a passage from a book (one experiment) and whispering the same passage (one experiment) did not produce any increased activity.

This difference between talking and reading a set passage is perhaps parallel to another effect that we observed. Thus, on one occasion (Fig. 3 B) W.W.P. forgot all about the experiment some time after the

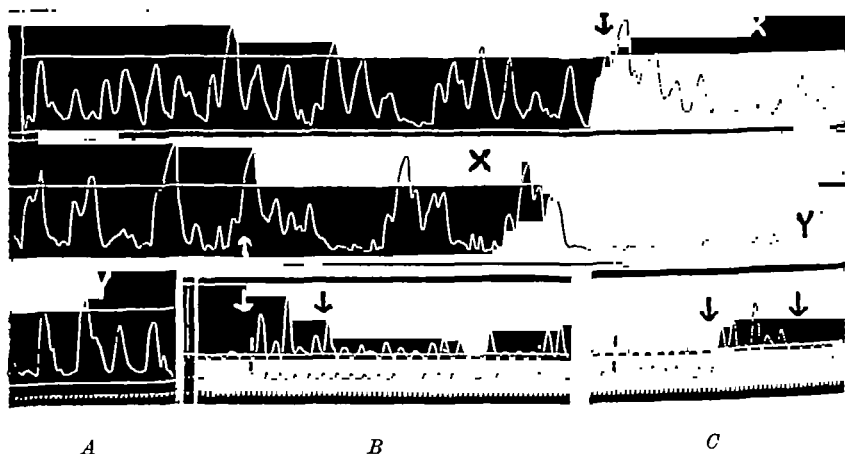


Fig. 3. Esophageal tracings. Horizontal lines 0 and 40 cm. Rubbing over sternum (A) calf (B, C) between the arrows. A, Exp. 6. Continuous tracing. Rubbing was painful at first X. At end of rubbing tingling of the skin grew less and disappeared at second X. It was noticed at Y again disappearing at second Y. Time marker sec. C, Exp. 7. In B and C, time marker  $\frac{1}{4}$  sec.

calf of his leg had been rubbed vigorously, and while the œsophageal movements were still being recorded by means of a bag containing 10 c.c. In fact he almost went to sleep. Nearly continuous activity was noticeable during this period of lack of attention, though the œsophagus had been quiet before. In another experiment (April 3rd, 1922) a bag containing 20 c.c. of air was in position at the lower end of W.W.P.'s œsophagus, which was active. Rubbing the sternum with lin. camph.

ammon. caused tingling. The activity of the œsophagus continued for nearly 3 min. During the experiment the subject swallowed several times. Immediately afterwards the experiment was repeated while the subject's attention was fixed on trying not to swallow. Rubbing which produced tingling and considerable pain caused practically no activity of the œsophagus at all in spite of the fact that the subject had to swallow once while he was being rubbed. This suggests that, when the attention is directed to some part concerned in the reflex, movements are to a large extent inhibited. It is possible that this occurs when the subject has to co-ordinate his voice with his breathing as in reading. Mosso and Pellacani(5) have demonstrated that the contraction of the bladder is to some extent under the control of the will, and the same may be the case with the œsophagus.

*Cutaneo-œsophageal reflexes.*

These reflexes were investigated by means of bags containing from 10 c.c. to 30 c.c. air. In these experiments, seventeen in all, pain was not felt; but sometimes there was enough air in the bag to produce vigorous activity on the part of the œsophagus. In the experiments the skin over the sternum was stimulated by applying a turpentine stupe or mustard leaf plaster, by rubbing with lin. camph. ammon. B.P., or by pinching or rubbing with cotton wool or the bare hand.

The type of response was independent of the methods of stimulation employed; it was as marked when the sternum was rubbed with cotton wool or the bare hand as when pungent liniment was used or a turpentine stupe applied. It was more marked when the subject did the rubbing himself. It varied to some extent with the activity of the œsophagus immediately preceding. When the œsophagus was quiet, as was usually the case with 10 c.c. in the bag, stimulation caused immediate activity which tended to die down after stimulation or during the stimulation itself, if this was prolonged. When the œsophagus was already active, stimulation sometimes caused a decrease in activity; at other times the activity might at first remain unaltered, or only the type of contraction be changed; but in these cases stimulation usually caused the activity to die down in the end. In three cases the activity was not obviously modified.

Increased activity of the œsophagus was indicated by the increased frequency and greater height of the contractions and by a simultaneous rise in tonus. With great activity large multiple contractions were seen. Emphasis is laid on the fact that the activity caused by rubbing is by no

means specific. In fact a tracing was sometimes obtained which was indistinguishable from that due to what was apparently spontaneous activity. On several occasions immediately after the rubbing was finished a contraction of the œsophagus was observed. There are examples of this in Figs. 3 B and 4 A.

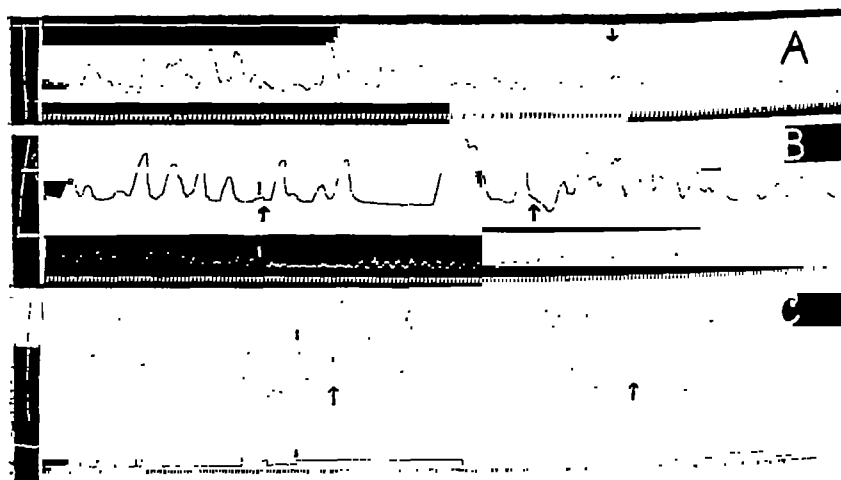


Fig. 4. Esophageal tracings. Horizontal lines 0 and 40 cm. Rubbing over sternum between the arrows. A, Exp. 5. B, Exp. 8. C, Exp. 10. Descent of lever indicates pain.

*Exp. 5.* (Fig. 4 A.) May 22nd, 1922. The subject was a medical student inclined to attacks of indigestion. He had just been through an experiment with 40 c.c. air in the bag, and this had caused slight pain in the back. Air was removed until the bag contained 15 c.c. and a record was obtained from the œsophagus which showed some activity. On rubbing the sternum with lin. camph. ammon. the activity continued for 22 sec.; it then decreased and after the rubbing the œsophagus became quite quiet, showing only respiratory variations.

A remarkable result was obtained with a very active œsophagus, which is described at length, because of its bearing on the counter-irritation experiments of the next section.

*Exp. 6.* (Fig. 3 A.) April 3rd, 1922. Subject E.P.P. Bag in lower œsophagus contained 30 c.c. During the preliminary 115 sec. the œsophagus was very active but the diastolic pressure was comparatively low and no pain was felt. Its activity was very similar to that on May 22nd, which was shown in Fig. 8 of Part I. The effect of rubbing the sternum with lin. camph. ammon. was to produce at the beginning a big multiple contraction showing a rise of diastolic and systolic pressure. This was followed by a number of rather small contractions. Some time after the rubbing had become painful the contractions again gradually became larger, and were now separated by increasing intervals of comparative quiescence. This continued after rubbing was stopped, and while tingling was being felt. 20 sec. after the end of the tingling the œsophagus became absolutely quiet for 39 sec. and

this was followed by further activity which coincided with a slight recrudescence of the tingling.

It is clear that when the œsophagus is active cutaneous stimulation causes periodic waxing and waning of its movement, so that the tracing resembles that of rather irregular Cheyne-Stokes breathing. Less marked examples of the same thing occurred in other experiments of this series.

The effect of rubbing the skin at a distance was also investigated. For this purpose the calf of the leg was chosen. There were six experiments, five without liniment and one with lin. aconiti (B.P.). In these experiments the bag did not contain more than 15 c.c. air. The same results were obtained as when the sternum was rubbed.

*Exp. 7.* (Fig. 3 c.) Subject W.W.P. June 1st, 1922. Bag in lower œsophagus contained 10 c.c. Immediate effect of rubbing the right calf with cotton wool was to produce activity during the rubbing with a slight increase of diastolic pressure to begin with. The movements were very similar to spontaneous movements which had occurred previously, and the same kind of activity was also observed some time subsequent to the rubbing. During the rubbing the bag produced a noticeable sensation in the throat.

Similar results were obtained in the other experiments. On the other hand, pinching the calf produced no effect in two experiments on E.P.P., and combing the hair vigorously enough to produce some pain caused no effect on W.W.P.'s œsophagus in one experiment.

### *Pain and counter-irritation.*

By counter-irritation is meant stimulation of the skin in order to allay pain produced in some internal organ. In our experiments pain was produced by filling a bag in the œsophagus with air nearly to its full capacity, and counter-irritation was carried out either by rubbing the skin over the sternum with cotton wool or a dry sponge, by rubbing with lin. camph. ammon. or applying a turpentine stupe. Seven experiments were carried out in all, four on E.P.P. and three on W.W.P. Results similar to those in *Exp. 7* might be expected. So far as the periodicity is concerned this was the case.

*Exp. 8.* (Fig. 4 b.) May 18th, 1922. Subject E.P.P. The bag contained 40 c.c. air, and its end was 12 in. below the teeth. The sternum was rubbed with lin. camph. ammon. for 98 sec. Continuous retrosternal pain was felt before rubbing, but no pain was felt during rubbing or for some time afterwards, and there is a note that the rubbing felt "comforting."

The tracing shows that the movements became periodic, but a new feature is present since the tonus shows a tendency to fall steadily. Thus, before the rubbing, the lowest diastolic pressure was 20 cm. and at the first period of waning 59 sec. from the beginning of rubbing it was 18 cm. At the next period of waning 45 sec. later, i.e. after the rubbing was over,



and pain was felt again, but was less than before; then contractions began again. After removal of the stupe tingling of the skin was noticed.

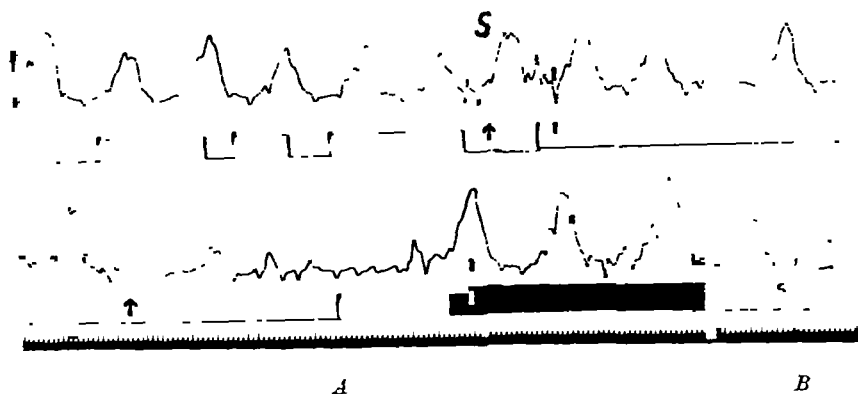


Fig. 5. Exp. 9. *A*, Continuous oesophageal tracing. Turpentine stupe applied between the arrows. *S*, Swallow. Owing to a leak in the piston recorder there is a gradual slight fall in the tracing, and no absolute pressures are given. Rise of lever—pain. *B*, End of previous tracing. A pressure difference of 40 cm. is shown.

In this experiment the period of increased activity came first on applying the stupe, which is the converse of Exp. 8.

In the next experiment there was no fall in diastolic pressure at all, but an obvious increase in contractions.

Exp. 10. (Fig. 4 c.) April 26th, 1922. Subject E.P.P. Bag in lower oesophagus contained 45 c.c. The sternum was rubbed with cotton wool for 97 sec. The oesophagus was very active all the time, but the activity increased during the rubbing. The diastolic pressure rose from 19 cm. before rubbing to 20.7 cm. 32 sec. from the beginning of rubbing, and was 19.7 cm. 74 sec. later just after rubbing had finished. At first retrosternal pain (shown by descent of lever) was felt frequently between the contractions; it was almost completely relieved during rubbing and reappeared immediately afterwards. The latter is only to be expected, since the usual fall of tonus which persists for some time after the counter-irritation failed to appear.

What was the reason of this failure?

We think it likely that the previous activity of the oesophagus was responsible, since, with prolonged stimulation, caused by the presence of the bag, the postural adaptation may reach its maximum so that cutaneous stimulation can have no further effect. Thus in Exp. 8, 7 min. from the beginning, the diastolic pressure was 20 cm.; 15–20 min. later it had fallen to 15.3 cm. even though the volume in the bag had been increased from 40 to 50 c.c. Further, counter-irritation after 7 min. caused a fall of 6.5 cm. in diastolic pressure, while 15 to 20 min. later

counter-irritation when again carried out only caused a fall of 1.3 cm. In Exp. 9 where the diastolic pressure actually rose, observations on the œsophagus had been carried out for 29 min. before counter-irritation.

Experiments on W.W.P. showed the same thing. After  $9\frac{1}{2}$  min. of œsophageal activity due to 45 c.c. in the bag counter-irritation caused a maximal fall of 3.8 cm. When the counter-irritation was repeated after another 7 min. of œsophageal activity, there was a rise in diastolic pressure.

Summarising our seven experiments on counter-irritation in all of which pain was relieved, it was found that in five there was a fall of diastolic pressure, while in four there was at some time or other an increase in contractions; in one experiment neither of these features were present, so that our theory obviously does not provide a complete explanation of the relief of pain, nor does it explain why this occurred at the very beginning of counter-irritation before the fall in diastolic pressure took place. A third factor may be the sensation produced on the skin by the rubbing which temporarily diverts attention from the sensation felt in the œsophagus as described in the section on gastro-œsophageal reflexes.

Cutaneous stimulation commonly produces a sensation of tingling in the skin of the subject. We have already observed in Exp. 6 that this tingling accompanied the period of increased œsophageal activity. Periods of tingling were noticed four times in three experiments of the present series. Three times the tingling was associated with increased activity on the part of the œsophagus. This is suggestive of some inter-relationship. Possibly the tingling sensation sometimes determines the time of the waxing phase of the rhythmic œsophageal activity.

The effect of cutaneous stimulation on visceral excitability has been observed in the "mass-reflex" described by Head and Riddoch(7). In patients with complete division of the spinal cord in the dorsal region, rubbing the skin of the lower limbs causes evacuation of the bladder with forced movements of the legs, etc. Danielopolu, Radovici and Carniol(8) have published tracings of large single contractions of the bladder which followed stimulation of the skin in a patient suffering from spastic paraplegia due to disease of the spinal cord. No experiments on normal people are given. From our observations on normal subjects it is evident that the results are not so stereotyped as in the mass reflex, and they depend on various conditions such as attention, length of previous stimulation, etc.; the main difference would appear to be that in the normal cutaneous stimulation is not invariably followed by

a single big contraction. Very often there is an increase of the small contractions with rise of systolic and diastolic pressure, whereas when the tonus is high enough to cause pain stimulation may produce a rhythmic activity with a fall in diastolic pressure and in some of our experiments, *e.g.* Fig. 4 B, the periodicity actually began with a waning phase.

#### CONCLUSIONS.

(1) Nausea which is defined as a sensation felt at the back and bottom of the throat is due to tension exercised on specific end organs in the œsophagus, especially its upper part. It is relieved either by peristaltic contraction, or increase of posture. When nausea is painful, it is suggested that the nausea end organs and the pain endings are excited simultaneously. Nausea is closely related to the sensation of "globus", but is different in quality.

(2) "Sinking"—a sensation felt in the abdomen—is produced in the stomach and is analogous in every way to nausea.

(3) The reflex effect on the œsophagus of distending the stomach with air is variable, since it depends on the activity of the œsophagus at the time.

(4) The smell of ammonia produces œsophageal activity. Swallowing iced water abolishes it. The activity of the œsophagus is greater when the subject is not paying attention to the experiment.

(5) Stimulation of the skin over the sternum or the calf causes in an inactive œsophagus activity which tends to die down. When the œsophagus shows slight activity, stimulation may make it quiet.

(6) When the œsophagus is very active cutaneous stimulation causes the movement to become periodic. When the tonus is high and pain is present counter-irritation causes in addition a lowering of diastolic pressure with marked relief of pain; it is suggested that its therapeutic value depends mainly on this fact, and to a smaller degree on the increased frequency of contraction in the active phase. When owing to long stimulation the posture of the œsophagus has reached its maximum counter-irritation has no effect on the tonus or may even cause a rise.

(7) The sensation of tingling is usually associated with a phase of œsophageal activity.



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## EXCITATION OF BENT NERVE.

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### INTRODUCTION.

IN a recent publication<sup>(1)</sup> an attempt was made to bring under a mathematical formula the changes in the threshold for nervous excitation which result from certain alterations in the position of the electrodes. The formula was derived from the following assumptions:

(a) The nerve is regarded as a cylinder with a relatively resistant sheath and a relatively conducting core.

(b) Nernst's assumptions regarding the dependence of excitation upon ionic concentration are accepted.

(c) Excitation is brought about by the current leaving the nerve through the cylindrical sheath, which thus constitutes Nernst's membrane.

(d) For stimulation with constant currents of fixed duration excitation will occur at a given point in the sheath when the current leaving at that point attains a certain fixed value.

The results of the mathematics were found to be in close accord with the experimental observations, namely the relation between threshold and interpolar length, and between threshold and angle between current and nerve. The foregoing assumptions therefore receive considerable support. But the whole of this analysis would be enormously strengthened if it could be shown that, without any multiplication of hypotheses, these assumptions would account for a new and wider range of observations.

The investigations in this paper, therefore, treat of a more complicated form of stimulation, and it is found that in every case there is a good agreement between observation and theory.

The mathematical treatment in this paper becomes rather technical in places, and so it was thought well to include it as an appendix at the end of the paper. The results of the mathematics can then be quoted in the text to compare with the experimental observations, and reference given to the place in the appendix where the result is obtained.

### THRESHOLD FOR BENT NERVE WITH DIFFERENT ANGLES OF CURRENT.

In the previous paper<sup>(1)</sup> a stretch of nerve was immersed in a solution and exposed at various angles to a current flowing through the solution in parallel lines. It was shown that, when the nerve was straight, the relation between threshold and angle, plotted in polar coordinates, gave two parallel straight lines. When, however, the nerve was bent by pulling the centre a little to one side, the lines became concave to the origin, and quite changed their shape, as is shown in Fig. 6 of that paper. This rough experiment was sufficient for the matter in hand, namely to trace an error in the experiments with a straight stretch, but it seemed of interest to see what form the plotted curve assumed when the nerve was bent more accurately in some manner. The simplest cases to take are when the exposed stretch consists of two straight limbs of equal length bent in the horizontal plane at various angles.

*Apparatus.* The trough mounted on a turn-table, and the electrical apparatus, were the same as in the experiments in the former paper (Fig. 4), the only change being in the part which held the nerve. This is shown in Fig. 1.

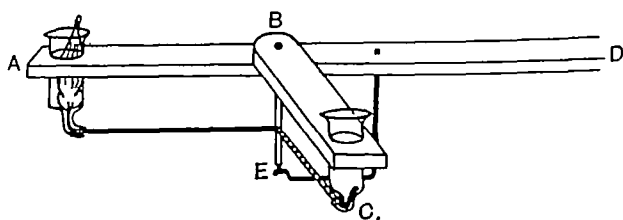


Fig. 1. Arrangement for holding the nerve.

The stand *AD* is held fast at *D*, and kept fixed throughout the experiment. The end *A* holds a glass tube containing the muscle (frog's gastrocnemius), and the sciatic is passed through the lower end of the tube, which is drawn fine and bent horizontal, pointing towards *D*. At *B*, a distance of 15 mm. from the centre of the tube *A*, is pivoted the movable arm *BC*, holding at *C* a tube similar to *A*, and equidistant from *B*. The horizontal part of the capillary end of *C* points towards *B*. The upper ends of two threads are attached to the pivot *B*, and the lower ends to *E*, a point vertically below. *E* is capable of executing a slight vertical movement, thus altering the tensions on the threads. First they are slackened and the nerve passed between them, then they are tightened

and hold the nerve in position. The central end of the nerve is passed into the tube *C*. On the arm *BC* is mounted a protractor (not shown) with its centre over the pivot *B* so that the angle *ABC* may be read direct. This is the angle at which the nerve is bent and it may be altered at will by simply moving round the arm *BC*.

*Experiment.* A frog's sciatic-gastrocnemius preparation was excised and a thread tied to the central end of the nerve. This thread had already been introduced into the apparatus in the position where the nerve was to go, and so the nerve was brought into position by gently drawing the free end of the thread from the upper opening of tube *C*. The preparation was then placed in the trough so that the direction *AB* was perpendicular to the line of current flow when the turn table was at zero. The angle between the two limbs was then set at the

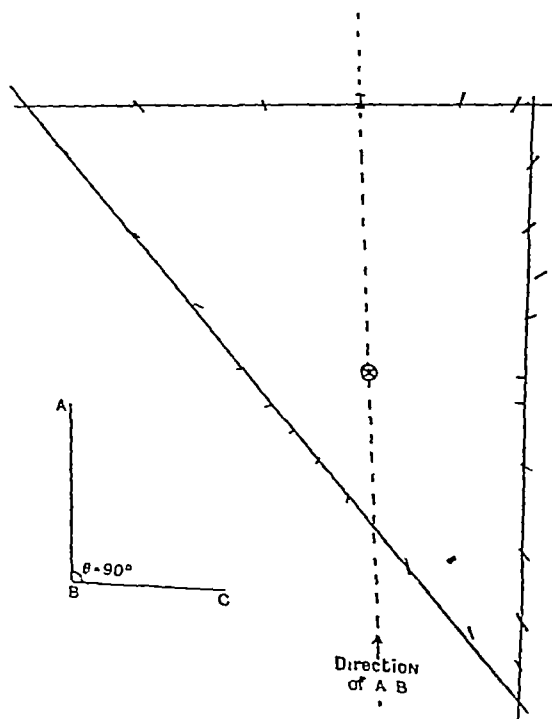


Fig. 2. Relation between threshold and angle which current makes with nerve bent at right angles.

required value and the preparation left for at least an hour to attain a steady state. As in the former experiments the stimulus was a constant

current delivered by a Lucas pendulum; the duration in this case was about 0.001 sec.

*Results.* The results are plotted in polar coordinates as in the former experiments: the distance of the plotted point from the origin is proportional to the threshold strength: the angle that the line joining the point to the origin makes with the direction  $AB$  is the angle which the current makes with the peripheral limb  $AB$ . A typical curve is shown in Fig. 2 together with a diagram showing the position of the two limbs of the nerve.

For instance, if we require the threshold when the current flows parallel to the central limb and towards the bend, we draw from the origin a line parallel to  $CB$  and in the same direction, and note the point where it cuts the experimental curve. The distance of this point from the origin is proportional to the threshold.

It is clear that to a first approximation at least the results form a triangle enclosing the origin.

In every experiment which I have made there are always three sharp discontinuities between which the curves are smooth and approximately straight. The immediate suggestion is that the three smooth lines correspond to excitation at three different points in the nerve, and that the discontinuities represent a transition from one to another of these points.

The correctness of this suggestion is a matter of the first importance in the interpretation of the results, and so I thought it worth while to prove it by several methods.

#### (a) *Intuitive Analysis.*

The three points which suggest themselves as likely seats of excitation are the two extremities where the nerve enters the glass tubes and the bend in the middle. If the three regions of the curve represent stimulation at these three points, we should expect that each region would correspond to the range of angles where the point in question is at a negative potential to the rest of the nerve.

Thus the central end of the nerve should be stimulated most by a current flowing parallel to the central limb of the nerve and away from the bend. Similarly the peripheral end should be most stimulated by a current flowing parallel to the peripheral limb and away from the bend. It is seen that in precisely these two directions are the thresholds for two of the regions of the curve a minimum. Stimulation at the bend should occur when the current flows parallel to the bisector of the angle of the bend, and into the bend, since in this case the bend is cathodic

with respect to all other points in the nerve. The results show that this is also roughly the direction of the minimum threshold in the third region.

(b) *Mathematical Analysis.*

This is the same as the foregoing but put quantitatively. It will be considered later.

(c) *Variation of the Position of one Limb of the Nerve.*

If the peripheral limb is kept fixed and the position of the central limb is varied, we should expect that that part of the curve which we have already supposed to represent excitation at the peripheral end would be unchanged. Fig. 3 shows the result of an experiment to investigate

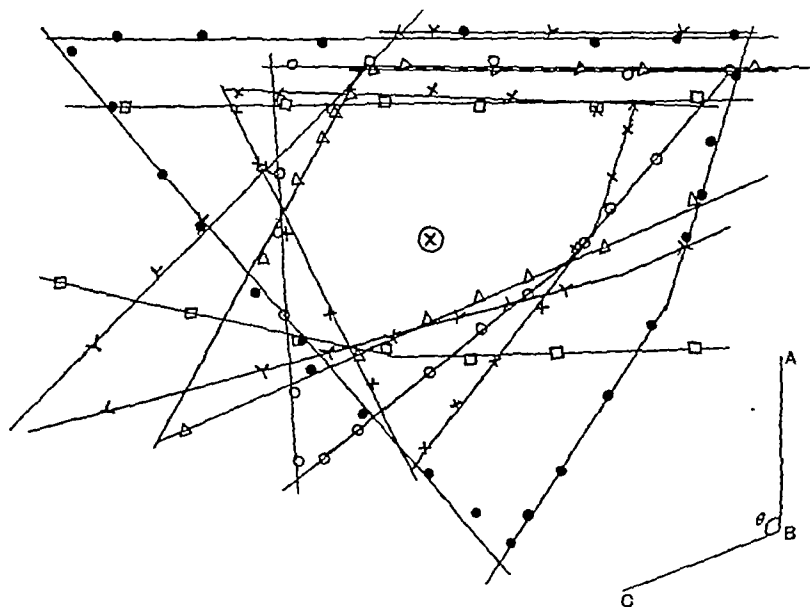


Fig. 3. Curves for nerve bent at various angles.

Order in which determinations were made:  $\square$ ,  $\theta=180^\circ$ ;  $\times$ ,  $\theta=120^\circ$ ;  
 $\triangle$ ,  $\theta=60^\circ$ ;  $\circ$ ,  $\theta=90^\circ$ ;  $+$ ,  $\theta=45^\circ$ ;  $\bullet$ ,  $\theta=135^\circ$ .

this. Here are plotted the results of six determinations made upon the same preparation with the central limb in various positions as indicated.

The results show quite clearly that when the angle at which the nerve is bent is altered, there is a change in the direction of two out of the three lines of the curve, but the third line remains unchanged in direction, and is only altered somewhat in threshold.

The line which is unchanged is seen to be that which we have already in (a) supposed to represent excitation at the peripheral end; this is still further confirmed by the results when the angle of bend was  $180^\circ$ . In this case we merely have a single stretch, and there is hardly room for doubt that here the line represents excitation at the peripheral end.

But furthermore, from symmetry we should expect that the line representing excitation at the central end would change its direction in each experiment by an angle equal to the change in  $\theta$ , the angle at which the nerve is bent. Of the remaining lines in each curve, one is seen to make with the line that we have just identified an angle of  $180^\circ - \theta$ , the other  $\theta/2$  (roughly). The former set of lines, which meet the identified line at its left end, are therefore to be recognised as representing excitation at the central end. This is confirmed by the case when  $\theta = 180^\circ$ , wherein this line becomes the lower parallel in the case of a single stretch and certainly represents excitation at the central end.

Moreover, the lines are seen to be those which in (a) we supposed to represent excitation at the central end.

The remaining line of the three is that which in (a) we assumed to represent excitation at the bend. Its direction is seen to be symmetrical with respect to the two ends of the nerve, and thus it is not unreasonable to suppose that it does represent excitation at the bend, the one point in the nerve which is thus symmetrical.

By "central end" of course is understood the central extremity of the exposed stretch, *i.e.* the point of entry into tube C, and not the actual cut end of the nerve. Similarly with the term "peripheral end."

#### (d) *Localisation by Heat.*

There is thus good evidence that two of the lines in the curve correspond to excitation at the two ends of the exposed stretch, but the evidence that the third line corresponds to excitation at the bend is not so good.

To verify this I made use of the fact that Ringer's fluid at a temperature of  $60^\circ\text{C}$ . permanently destroys the local excitability of a nerve, but the main structure is not much affected. The current may thus be supposed to distribute itself as before, but only the region of the nerve which has not been heated will be able to respond to excitation.

*Experiment.* The nerve was set up with its two limbs bent at a suitable angle, and the triangular curve determined in the usual way. Then the central end of the nerve was dipped into Ringer's fluid at  $60^\circ\text{C}$ . so that two-thirds of the central limb was immersed. After about two

minutes the nerve was withdrawn and replaced in the stimulating trough, and allowed to rest for half an hour for the destructive processes to subside. Then determinations were made as before. Again the nerve was dipped into Ringer's fluid at  $60^{\circ}\text{C}.$  this time until all but the peripheral end with half the peripheral limb were immersed. After two minutes the nerve was replaced in the trough, and after a delay of half an hour determinations were again made.

*Results.* The results are seen to be entirely confirmatory (Fig. 4).

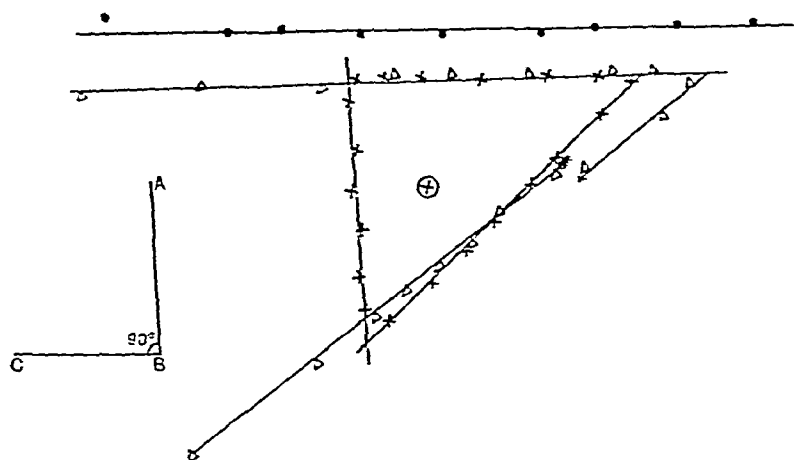


Fig. 4. Localisation by heat.

X, initial determination.  $\Delta$ , after heating C.  $\bullet$ , after heating B.

Initially a triangle was obtained in the usual way (crosses). After heating the central end of the nerve the side corresponding to this vanished, but the other sides were practically unchanged (triangles). After heating the bend, the side corresponding to this vanished too, showing that its presence depends upon the integrity of some place in the nerve not more than about 5 mm. from the bend.

(In the figure the side corresponding to excitation at the bend appears to have a discontinuity in the curve when the central end had been heated. This is illusory. One of the points was the first of the series of determinations, the other its repetition at the end. During the course of the experiment the threshold had altered from one to the other, due no doubt to the partial recovery from the injury being incomplete at the outset of the observations.)

The curves give good evidence also that the transition from one line to another of the triangle is merely due to the experimental selection of



the lowest threshold in every case. When what was the most excitable point is destroyed, the remaining points must be excited, and the regions of the curve corresponding to these are seen to stretch out indefinitely, continuous with the short regions first observed. This is also demonstrated with the experiments with alcohol shown in Fig. 5.

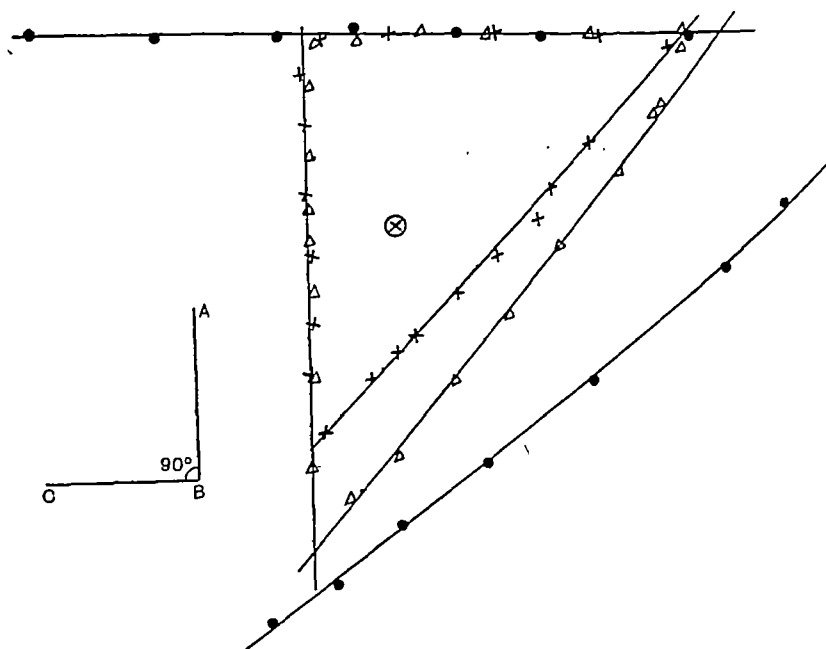


Fig. 5. Effect of application of alcohol to bend.  
X, initial determination. ●, 10 min. after application of alcohol. Δ, 20 min. later.

### (e) Localisation by Alcohol.

To confirm the results of the application of heat locally to the nerve, I tried the effect of local application of alcohol. The principle is somewhat similar, but with the difference that the change of excitability is reversible and gradual. This involves a considerable experimental difficulty because a strictly localised dose of narcotic, even if very strong, is almost completely washed out of the tissue in the half hour or so necessary for the determinations. Most things which prevent the washing out of the alcohol by the fresh solution prevent also the free passage of the stimulating current, but this experiment was made quantitatively possible by the discovery that gelatine in Ringer's fluid, to which is added about 30 p.c. alcohol, will greatly retard the washing out of the

alcohol from the nerve when it has "set" round the narcotised place. And since it has long been known that gelatine does not appreciably alter the conductivity of a solution in which it is made up, it will not appreciably alter the lines of current flow.

*Experiment.* The preparation was set up and the triangular curve obtained as usual. The nerve was then removed from the trough, and 30 p.c. alcohol in Ringer's fluid was applied to the bend, care being taken that it did not escape to other parts of the nerve. After about a minute, alcohol-gelatine-Ringer at room temperature but still liquid was applied to the same place and allowed to "set."

The nerve was then replaced in the trough and allowed to rest for ten minutes. Then the second set of determinations was made as fast as possible. After another interval of some 20 minutes the third set of observations was made.

*Results.* These also entirely confirm the earlier conclusions (Fig. 5). The first curve is the normal triangle (crosses). The second curve (dots), taken soon after the application of alcohol to the bend, shows no change in the line corresponding to stimulation at the peripheral end, a rise of threshold at the bend to about  $2\frac{1}{2}$  times the initial value, and the apparent complete inexcitability of the central end. Since, however, the central end has been no more narcotised than the peripheral end whose excitability is unchanged, the disappearance of the line corresponding to excitation at the central end must be interpreted differently. It is, in fact, due to a conduction block at the bend which prevents the propagated disturbance set up in the central limb from reaching the muscle and being indicated by it. The justification for this conclusion is derived from the third curve (triangles). It is here seen that long before the excitability of the bend has reached normal, the excitability at the central end has completely recovered. It is only necessary that the conduction block be so far lifted as to allow an impulse to pass, for the threshold for the central end to be revealed quite unaltered. The reason that the three lines corresponding to the bend are not parallel is because the threshold was diminishing during the course of the second and third determinations. The second curve (dots) was determined in a clockwise direction, the third curve (triangles) in the reverse direction, hence the opposite inclinations.

The three regions of the curve are thus seen to respond in three different and quite characteristic ways, and thus can once again be identified to confirm the former conclusions.

COMPARISON OF THE OBSERVATIONS WITH THE  
MATHEMATICAL RESULTS.

The mathematical treatment is given in the Appendix to this paper and the results only are quoted here. The capital letters refer to the section of the mathematics where the result quoted is obtained.

Now the mathematics are entirely based upon the assumptions made in the previous paper and summarised at the beginning of this one, and they merely give the quantitative results of the distribution of current in a conductor such as a nerve. In particular, the value of the current leaving the nerve through the sheath at any point is obtained, for this is the current which excites.

Now from the observations so far discussed there is very good evidence, first that they arise from three separate points on the nerve, second that these points are in the regions of the two extremities and of the bend, third that the three are quite independent so that in any position excitation will be elicited from that point where the threshold is least at the moment.

These three conclusions, which are entirely drawn from the experimental results, lend good support to the mathematical theory since they may be exactly deduced from the original assumptions.

It is found (*A*, *C*) that there are in the whole nerve stretch, three points such that the current leaving through the sheath at one or other of them is always greater than at any other point whatever, and hence excitation will always occur at one of these three points with a lower threshold than at any other. It is found, moreover, that the position of these points is exactly where we have supposed them, namely at the bend and at the points of entry of the nerve into the tubes. Finally, since the excitation is assumed to be dependent upon the current leaving the nerve locally, it will be quite independent of local changes of excitability elsewhere.

Since experiment and calculation agree as to the nature of the three portions of the curve obtained, it will be legitimate to go further and to calculate the exact form of the curves.

It is found (*B*) that each of the three portions should be a straight line, and hence the results should be strictly a triangle.

Some experiments (Figs. 2, 4, 5) gave this to a very good approximation, but some (especially the earlier ones) did not. The deviation, when it occurred, was always in the direction of a concavity towards the

origin. It is necessary to discover whether this effect when present indicates a fallacy in the theory, or an experimental error.

We may consider first the lines corresponding to stimulation at the ends of the nerve. The case here is very similar to that of a single straight stretch, and it was natural to suppose that the cause of the deviation in both experiments was the same, namely that the stretch was *not quite straight*. In confirmation of this it was found that if both the limbs of the nerve were kept stretched rather tightly, the two regions corresponding to excitation at the extremities were exactly straight (Fig. 6).

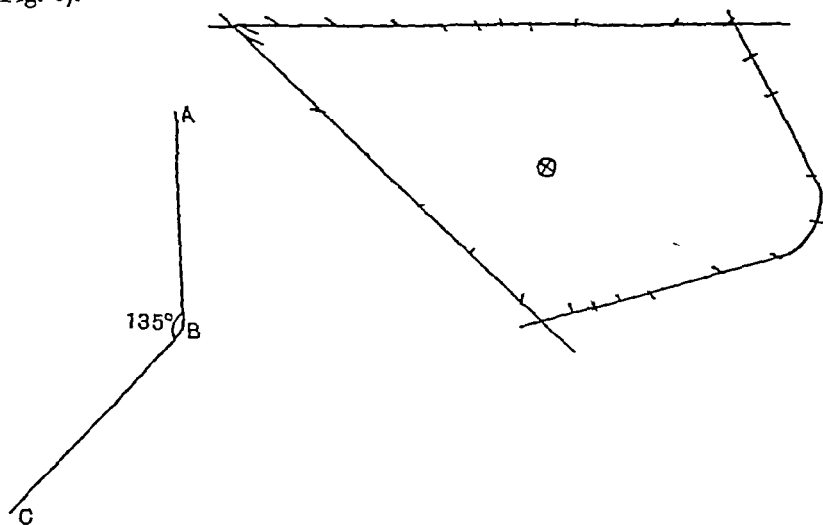


Fig. 6. Nerve constricted at bend.

Stimulation at the bend, however, cannot have the deviation explained in quite the same way. At first I thought that the explanation lay in the fact that the nerve did not bend at a point (as the calculation assumed) but round the arc of a small circle. To diminish this I bent the nerve round a fine silk thread and stretched both limbs. The results showed, however (Fig. 6), that this procedure, far from diminishing the deviation from a straight line, greatly increased it, and at the same time clearly defined the form of it. This region of the curve had split up into two straight lines deviating by a considerable angle: in the case shown this is greater than a right angle.

I observed the bend through a powerful dissecting microscope, and found that the inner edge was greatly compressed by the thread and the outer edge flattened by the pull of the nerve, so that it was constricted

exactly like a badly bent glass tube. It is evident that it is to this constriction that the anomaly was due.

I have calculated the effect of constricting a nerve fibre at the point where it is bent, and it is easy to show that the results in this case are two straight lines instead of one. The deviation between the lines is greater, the greater the constriction, reaching a maximum value equal to  $\theta$ , the angle of the bend.

This matter may be of interest in experiments investigating the action of striction, but it is beyond the scope of the present paper, and I have not thought it worth while to include the above calculation in the Appendix. The only present value of the observation is that on the one hand it indicates that not even these results are necessarily beyond the scope of the theory, and on the other, it confirms the suggestion that the deviation from a straight line is due to the constriction at the bend. Fig. 3 affords other examples of the region of the bend breaking up into two straight lines. In this experiment, again, the nerve was stretched, as was necessary if the peripheral limb was not to change position when the central limb was altered.

It thus appears that the condition for straight lines on two sides of the triangle (namely stretching of the nerve) is opposed to that for the third side. It is therefore a matter of some delicacy to adjust the exact degree of tension to realise all the conditions of the mathematics at once, but it is easy to verify the calculation at will either for the bend or for the extremities in any preparation by having the nerve loose or tight respectively, and in this way it is always possible to get a very good agreement between observation and calculation.

Since the experimental results have been shown to be strictly composed of straight lines, we may finally enquire whether the directions of them are also in conformity with the calculation.

From (C) we find that if  $\theta$  is the angle of the bend, the two lines of the results corresponding to excitation at the ends meet at an angle of  $(\pi - \theta - \cdot 1 \sin \theta)$  measured in radians.

Thus instead of this angle being supplementary to  $\theta$  as was supposed in the intuitive argument above, we find that it is less than this by  $\cdot 1 \sin \theta$  radians which amount to

4°	when	$\theta = 90^\circ \pm 45^\circ$ ,
5°	„	$\theta = 90^\circ \pm 30^\circ$ ,
6°	„	$\theta = 90^\circ$ .

When this result is compared with those curves which do not exhibit the striction phenomenon, the angle in question is seen to be always a

little less than  $180^\circ - \theta$ , and the amount less is very close to the required values. The matter cannot be pressed too far, for the method of bending the nerve admits of a zero error of one or two degrees in the reading of  $\theta$ .

With regard to the third side of the triangle. when straight, it appears from (C) that it should meet the other two lines at approximately equal angles. The difference of angles should not exceed  $\theta/15$ . In Fig. 1 this value is somewhat exceeded, but in Figs. 4 and 5 the results are well within the limits.

The more the nerve is constricted at the bend. the less will each extremity be influenced by the condition of the remoter half of the nerve. and the more will the angle first considered approximate to  $180^\circ - \theta$ . This is seen to be actually the case in those curves exhibiting the striction effect. All the curves which indicate great striction give an angle sensibly equal to  $180^\circ - \theta$ , while all the curves showing no striction give an angle sensibly less.

Within the limits of the experiments, therefore. there is a good accord between the calculated and observed values of the angles of the various lines, and the theory put forward in the previous paper is, without any further hypothesis, found to be justified quantitatively in its three main deductions:

- (a) The results show three different regions corresponding to the three definite places on the nerve discussed above.
- (b) For each region the results. plotted in polar coordinates, are straight lines.
- (c) The direction of each line is as predicted.

## APPENDIX.

### MATHEMATICAL TREATMENT OF CURRENT DISTRIBUTION.

In the former paper<sup>(1)</sup> the distribution of current in a nerve was calculated for the simple case where the ends of the nerve were in equipotential solutions, and the intermediate region was exposed to a uniform potential gradient. Since then I have been able to make a considerable advance in the mathematical treatment, and the formula which will be derived in the sequel is superior to the previous one both with regard to its very wide scope, and to the great ease with which it can be applied in most practical cases. It has, moreover, the advantage that it may be deduced from first principles in a few lines.

*Definitions.*

These are the same as in the former treatment, but we may mention

*Analytical Unit of Length* is the length of cylinder which has the same resistance to currents passing axially down the core as it has to currents passing radially through the sheath, and this resistance is defined as the *Analytical Unit of Resistance*.

$x$  = length measured along the nerve in analytical units.

$V$  = potential of core at any point  $x$ .

$U$  = potential applied to outside of sheath at  $x$ .

$$U' = \frac{\partial U}{\partial x^2}, \quad U'' = \frac{\partial^2 U}{\partial x^2}.$$

Then current leaving the core at  $x$

$$= \frac{\partial^2 V}{\partial x^2} = V - U.$$

*Analysis.*

Now the complete primitive of this differential equation, as can easily be verified by differentiation, is

$$2V = 2U + e^{-x} \int_{-\infty}^x e^x U'' dx + e^x \int_x^{\infty} e^{-x} U'' dx + ae^{-x} + be^x. \quad \dots\dots\dots(1)$$

The arbitrary constants may be evaluated from the following assumptions. Both ends of the nerve are in equipotential solutions, the end  $x = -p$  is closed by a membrane of resistance  $r_1$ , the other end  $x = q$  by a membrane of resistance  $r_2$ . Then

$$\left( \frac{\partial^2 V}{\partial x^2} / \frac{\partial V}{\partial x} \right)_{-p} = r_1, \quad \left( \frac{\partial^2 V}{\partial x^2} / \frac{\partial V}{\partial x} \right)_q = -r_2.$$

From (1) we obtain

$$\left( \frac{\partial^2 V}{\partial x^2} \right)_{-p}, \quad \left( \frac{\partial V}{\partial x} \right)_{-p}, \quad \left( \frac{\partial^2 V}{\partial x^2} \right)_q, \quad \left( \frac{\partial V}{\partial x} \right)_q,$$

noting that

$$\left( \frac{\partial U}{\partial x} \right)_{-p} = 0 = \left( \frac{\partial U}{\partial x} \right)_q.$$

Also since  $U$  is assumed to be constant between  $x = -\infty$  and  $-p$ , and between  $x = q$  and  $\infty$ ,

$$\int_{-\infty}^{-p} e^x U'' dx = 0 = \int_q^{\infty} e^{-x} U'' dx.$$

$$\therefore a = \frac{r_1 - 1}{r_1 + 1} e^{-2p} \left[ b + \int_{-\infty}^{\infty} e^{-x} U'' dx \right], \quad b = \frac{r_2 - 1}{r_2 + 1} e^{-2q} \left[ a + \int_{-\infty}^{\infty} e^x U'' dx \right].$$

$$\begin{aligned} \therefore a + b = & \frac{r_1 - 1}{r_1 + 1} e^{-2p} \int_{-\infty}^{\infty} e^{-x} U'' dx + \frac{r_2 - 1}{r_2 + 1} e^{-2q} \int_{-\infty}^{\infty} e^x U'' dx \\ & + \frac{r_1 - 1}{r_1 + 1} \cdot \frac{r_2 - 1}{r_2 + 1} e^{-2(p+q)} \left[ a + b + \int_{-\infty}^{\infty} (e^x + e^{-x}) U'' dx \right]. \end{aligned}$$

But since  $p + q = \text{total length of nerve} > 6 \text{ units}$

$$e^{-2(p+q)} < 1/160,000.$$

The last term is therefore negligible, and substituting in (1), where  $x$  is put zero, we obtain

$$2 \left( \frac{\partial^2 V}{\partial x^2} \right)_{x=0} = 2(V-U)_{x=0} = \int_{-\infty}^0 e^{-x} U'' dx + \int_0^{\infty} e^{-x} U'' dx \\ + \frac{r_1-1}{r_1+1} e^{-2p} \int_{-\infty}^{\infty} e^{-x} U'' dx + \frac{r_2-1}{r_2+1} e^{-2q} \int_{-\infty}^{\infty} e^{-x} U'' dx. \dots\dots(2)$$

Now in all the cases to be considered  $U$  is made up entirely of straight lines, hence  $U''$  vanishes except where two lines meet, and this greatly simplifies the integration.

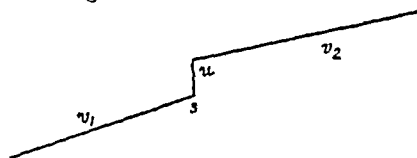


Fig. 7.

If  $U$  is the function shown, such that for values of  $x < s$ ,  $U' = v_1$ ; for  $x > s$ ,  $U' = v_2$ .

Also if  $U_{s+\Delta x} - U_{s-\Delta x} \rightarrow u$  when  $\Delta x \rightarrow 0$ ,

then  $\int_{s-\Delta x}^{s+\Delta x} e^{-x} U'' dx = e^{-s} (v_2 - v_1 + u)$ ,  $\int_{s-\Delta x}^{s+\Delta x} e^{-x} U'' dx = e^{-s} (v_2 - v_1 - u)$ , .....(3)

as can be proved by twice integrating by parts.

It thus becomes a matter of the greatest simplicity to integrate (2) by merely applying (3) in turn to each junction of the lines within the range of the integral.

#### (A) Localisation of Excitation.

The nerve is stimulated by the current leaving it,  $\frac{\partial^2 V}{\partial x^2}$ , and this may be calculated for any function  $U$ , at the point  $x = 0$  from (2). Since the outer limits of the integrals are infinite, we may change the origin, and cause the point  $x = 0$  to coincide with any place on the nerve whatever. In practice, however, we are interested only in that point where the excitation is greatest. For a nerve in a uniform condition this will be the place where  $\frac{\partial^2 V}{\partial x^2}$  is greatest. We proceed to show that if  $U$  is linear between  $x = s_1$  and  $x = s_2$ , then  $\left| \frac{\partial^2 V}{\partial x^2} \right|$ , either at  $s_1$  or at  $s_2$  will be greater than at any intermediate point.

For if we consider (1) it immediately appears that whatever value  $x$



assumes within the range under consideration, the coefficients of  $e^x$  and  $e^{-x}$  are unaltered. We thus obtain

$$2 \left( \frac{\partial^2 V}{\partial x^2} \right)_x = A e^x + B e^{-x} \equiv C \sinh \overline{x + \kappa}, \quad \text{or} \quad C \cosh \overline{x + \kappa},$$

where  $\kappa$  is a constant, and these hyperbolic functions in any range have the value at one extreme numerically greater than all other values in the range.

We thus conclude that the excitation need only be considered at the junctions of the lines which compose  $U$ .

(B) *Application to Experiment with Bent Nerve.*

In the previous paper it was shown that the diameter of a single nerve fibre is so small, and the resistance of its sheath so large, that no sensible error would be introduced in assuming that in any transverse section the potential was uniform over the whole of the core, and over the outside of the sheath. We are, in fact, justified in speaking of the potential of the core, or the potential applied to the sheath, at a certain distance along the axis.

Thus if  $ABC$  is the nerve bent at an angle  $\theta$ , and if the lines of current

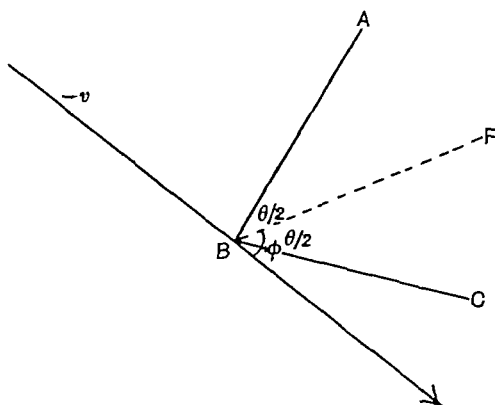


Fig. 8.

flow are in the direction of the arrow, making an angle  $\phi$  with the bisector  $BF$  of the angle  $ABC$ , then the effect on the nerve is found by resolving parallel to the two lines  $AB$ ,  $BC$ .

If the potential gradient in the direction of the arrow is  $-v$ , then the value in the direction  $AB$  is  $(v \cos \overline{\phi + \theta/2})$ , and in the direction  $BC$  it is  $(-v \cos \overline{\phi - \theta/2})$ .

The whole nerve stretch is thus exposed from the end to  $A$  in an equipotential region.

$A$  to  $B$  in a region of gradient  $v \cos \overline{\phi + \theta/2}$ .

$B$  to  $C$  in a region of gradient  $-v \cos \overline{\phi - \theta/2}$ .

$C$  to the end in an equipotential region.

$AB = BC = s$ .

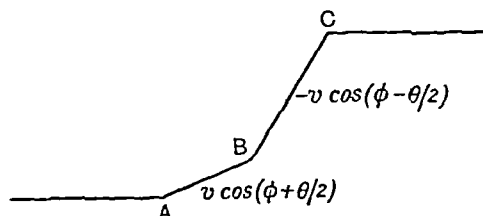


Fig. 9.

Now in (A) it was shown that to calculate the threshold excitation we need only consider the points  $A$ ,  $B$  and  $C$ , thus we obtain the theoretical result simply by applying formula (2) to the function  $U$  shown in Fig. 9, where the origin is made to coincide successively with  $A$ ,  $B$  and  $C$ .

To obtain numerical values we note that  $s \approx 3$  units,  $q > 6$  units, hence  $e^{-s} = 0.05$  and terms in  $e^{-2s}$  and in  $e^{-q}$  are negligible.

After this simplification we obtain

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_A \propto 0.95v \cos \overline{\phi - \theta/2} - 0.05v \cos \overline{\phi - \theta/2}, \quad \text{similarly} \quad \left(\frac{\partial^2 V}{\partial x^2}\right)_C;$$

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_B \propto -v \cos \overline{\phi - \theta/2} \left[1 - e^{-s} - \frac{r_1 - 1}{r_1 + 1} e^{-2s + s}\right] - v \cos \overline{\phi - \theta/2} \left[1 - e^{-s} - \frac{r_1 - 1}{r_1 + 1} e^{-2s + s}\right],$$

where the factors of proportionality do not involve  $\phi$ .

Making use of the trigonometrical identity

$$a \cos \overline{\phi - \theta/2} + b \cos \overline{\phi - \theta/2} \equiv c \cos \overline{\phi - \psi/2},$$

where

$$c^2 = a^2 + b^2 + 2ab \cos \theta, \quad \tan \psi/2 = \frac{a-b}{a+b} \tan \theta/2;$$

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_A \propto v \cos \overline{\phi - \psi/2}, \quad \text{where} \quad \psi/2 = \theta/2 - 0.05 \sin \theta \text{ radians (approx.).}$$

(C) Thus the experimental results, plotted in polar coordinates  $v, \phi$ , should give two straight lines corresponding to stimulation at the two extremities  $A$  and  $C$ , and these lines meet at an angle  $(\pi - \theta - 0.1 \sin \theta)$  in circular measure.

Analysing in a similar way the expression for  $\left(\frac{\partial^2 V}{\partial x^2}\right)_B$  we obtain, when  $\theta < 140^\circ$ ,

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_B \propto -v \cos \overline{\phi + \psi}, \quad \text{where} \quad \psi < \pm \theta/30.$$

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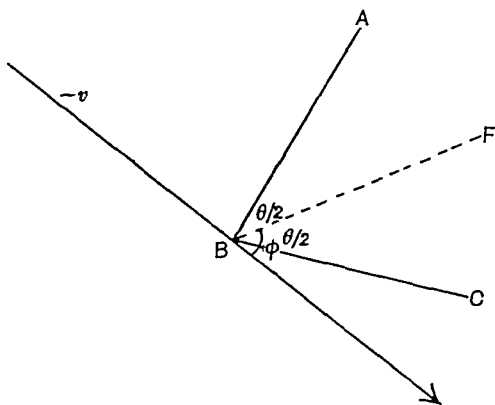


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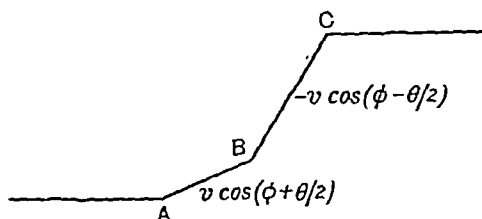


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Analysing in a similar way the expression for  $\left(\frac{\partial^2 V}{\partial x^2}\right)_B$  we obtain, when  $\theta < 140^\circ$ ,

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_B \propto -v \cos \overline{\phi - \psi}, \quad \text{where} \quad \psi < \pm \theta/30.$$

The third side of the triangle is therefore also straight, and within narrow limits its direction is perpendicular to the bisector of the angle of the bend.

#### SUMMARY.

(1) A nerve consisting of two straight stretches bent at an angle was stimulated by a current flowing in parallel lines, and for various values of this angle the relation was found between the threshold and the angle between current and nerve.

(2) Excitation was found to occur at one of three points in the nerve, namely at the bend or at one or other extremity of the exposed region, and the excitatory process at any one of these points was found to be quite independent of the process elsewhere.

(3) The results, plotted in polar coordinates, form a triangle each side of which corresponds to excitation at one of the three points mentioned in (2).

(4) All the results obtained can be deduced theoretically without any new hypothesis from the assumptions made in a previous paper<sup>(1)</sup> and summarised at the beginning of this one.

(5) In the Appendix the mathematical treatment is developed in a form very suitable for application to most questions of the dependence of threshold upon the arrangement of the electrodes.

In conclusion I wish to express my thanks to Dr Adrian for his valuable criticism.

I am also indebted to the trustees of the George Henry Lewes Studentship, and the donor of the Stokes Studentship.

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1. W. A. H. Rushton. *This Journ.* 63. p. 357. 1927.

## THE EFFECT OF INSULIN ON ACETONURIA.

By J. H. BURN AND H. W. LING.

*(From the Pharmacological Laboratory of the Pharmaceutical Society of Great Britain, London.)*

THE experiments to be described were begun two years ago in order to obtain further information on the part played by the secretion of the posterior lobe of the pituitary body in fat metabolism. Coope and Chamberlain(1) had already shown that injections of pituitary extract caused a rise in the percentage of fat in the liver of rabbits, and the suggestion presented itself of investigating the relation of pituitary extract to the formation of acetone bodies. Observations were first made on the acetonuria occurring in the dog and in the cat when these animals were fed on a fat diet. The finding of Geelmuyden(2) that the acetonuria so produced is very small in amount was confirmed, and attention was turned to the more regular excretion which Wigglesworth(3) first recorded in the rat.

In a preliminary note(4) we have already stated that injections of pituitary extract greatly diminish or inhibit the ketonuria occurring in the rat on the second and third days of a fat diet, in the months of May and June; further, that injections of adrenaline have the same action. Since this was published we have seen the papers of Raab(5), of Hirschhorn and Pollak(6), and of Anderson and Anderson(7).

Raab described a rise in the acetone bodies present in the blood of the dog following the injection of adrenaline. The changes he records, though definite, are not of such a magnitude as to suggest that the phenomenon is of great physiological importance. The work of Hirschhorn and Pollak on the other hand is much more impressive. They describe an increased output of acetone bodies following the injection of adrenaline (a) in normal human subjects on a carbohydrate free diet. (b) in pyrexial subjects on a low diet, (c) in patients suffering from vomiting of pregnancy. (d) in certain asthmatic patients, and (e) in diabetics. In all cases the rise in the acetonuria observed was striking. Finally Anderson and Anderson(7) have shown that in rats which have been kept for several days on a fat diet, or which are phlorizinised and fed on a carbohydrate-free diet, injections of adrenaline cause an

immediate rise in the output of acetone bodies. Their results are as clear and convincing as those of Hirschhorn and Pollak. The position therefore is that three independent investigations have shown that adrenaline can cause a rise in acetonuria in conditions of carbohydrate deprivation, while we have stated on the other hand that adrenaline inhibits acetonuria. For the present we do not propose to describe our experiments on pituitary extract and adrenaline in detail until we have had a further opportunity to investigate this discrepancy.

In the present paper experiments are described which demonstrate the seasonal variation in the acetonuria, the changes in the liver glycogen, and the action of insulin.

### *Methods.*

The No. 3 diet of Wigglesworth<sup>(3)</sup> has been used, consisting of butter, filtered when hot to remove traces of casein, and containing 3 p.c. of a salt mixture. The rats were put in a Hopkins metabolism cage in pairs of the same sex, and 24 hour samples of urine were collected. For estimating acetone bodies, instead of the distillation method used by Wigglesworth, we have used the gravimetric method of van Slyke<sup>(8)</sup>.

The rats were selected between the weights of 80 and 150 gm., and for the most part were males of about 120 gm. They were all animals from a Wistar strain bred in the Glaxo Laboratories.

### *Seasonal variation in the ketonuria.*

From March to July 1926 a degree of ketonuria was obtained of the same order as that described by Wigglesworth, whose experiments were carried out at the same time of the year. In the autumn and winter of 1926, however, the ketonuria was much less, and was too small to serve as a basis for the observations we wished to make. We were at a loss to account for the change until the paper of Cori and Cori<sup>(9)</sup> appeared in April 1927. These workers described a seasonal variation in the ketonuria of rats kept without food for 48 hours. They found that in the summer months there was about two to three times as great an excretion of acetone bodies as during winter. Our observations confirmed this suggestion, though the magnitude of the seasonal difference in our experiments considerably exceeded that recorded by Cori and Cori. When fresh observations were made in the spring of 1927, in which a greater ketonuria was recorded than in winter, there no longer remained any doubt of the existence of this unexpected phenomenon.

In Table I is recorded the average result of all the experiments done in each month of the year.

TABLE I.

Month	No. of observations	Mg. acetone per diem (two rats)						
		1st	2nd	3rd	4th	5th	6th	7th
Jan.	5	4	12	12	(17)	(6)	—	—
Feb.	2	3	5	7	—	—	—	—
March	1	4	4	7	4	2	2	—
April	2	3	17	32	43	18	6	—
May	5	3	36	67	(68)	—	—	—
June	5	8	41	51	38	(14)	(5)	—
July	10	2	26	36	22	14	(13)	—
Aug.	—	—	—	—	—	—	—	—
Sept.	{ 8*	7	27	22	15	11	14	10
	{ 4†	1	4	5	4	4	2	2
Oct.	3	2	4	5	6	—	—	—
Nov.	6	5	13	7	(15)	(8)	(7)	(6)
Dec.	3	2	6	5	—	—	—	—

\*=Males.

†=Females.

Brackets indicate that figure is average of smaller number of observations than stated in second column.

If the experiments be divided into two groups, in one of which is included those carried out from October to March, and in the other those carried out from April to July, there are 20 experiments in the first and 22 in the second group. The average third day excretion in the first group corresponded to 8 mg. acetone, while in the second it corresponded to 42 mg. acetone. The standard deviations of these two figures are 1.16 and 6.25 respectively, so that the difference between the two averages is statistically significant. (The ratio of the difference between the averages to the square root of the sum of the squares of the standard deviations is 5.5.)

Cori and Cori<sup>(9)</sup> investigated the effect of temperature changes on the ketonuria of starved rats and showed that the higher ketonuria of the summer months could not be produced in winter by putting rats for three weeks in a room at 28° C. In our experiments there has been no record of the temperature of the room in which the rats were kept, but there is no obvious wide variation. The September results are of interest because they suggest that female rats have already adopted winter behaviour in advance of the males; but very few observations on female rats have been made.

#### *Effect of successive periods of fat diet.*

In order to obtain evidence of the effect of any agent on the ketonuria, it was necessary to observe the behaviour of the same animals on two



occasions, separated by two or three weeks. It has been found that as a rule the ketonuria during a second period of fat diet is appreciably less than that during the first period. The experiment given in Table II is an example of this.

TABLE II.  
Two rats (♂), 120 grm. each.

Date when fat diet begun	Mg. acetone per diem			
	1st	2nd	3rd	4th
May 24th	17	70	43	68
June 20th	2	18	6	3

*The action of insulin.*

According to the time of the year at which the experiment is performed, there are three different responses to injections of insulin.

In Fig. 1 appears the record of an experiment carried out in September in which the pair of rats used were given a preliminary period

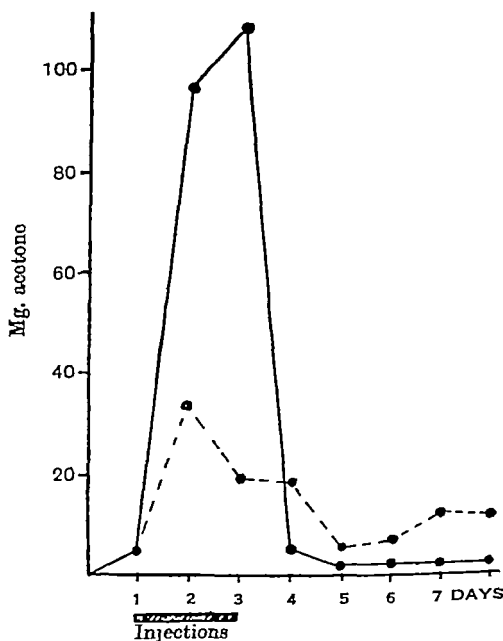


Fig. 1. Broken line shows excretion of acetone bodies by two rats in September. Continuous line shows excretion in same rats when injections of insulin were given. See text.

of fat diet (broken line) to determine the excretion of acetone bodies in the absence of any injection. After a rest of three weeks on a normal

diet. a second period of fat diet was begun, on the second and third days of which injections of insulin were made. The doses employed, per rat, were three doses of 0.4 units at 11 a.m., 1 p.m. and 5 p.m. and one dose of 0.3 units at 3 p.m. on the first day of injections, and three doses of 0.4 units at 1 p.m., 3 p.m. and 5 p.m. following an initial dose of 0.6 units at 11 a.m. on the second day of injections. The figure shows the striking rise in acetone body excretion which the insulin caused. It also shows the very rapid disappearance of the ketonuria when the injections were stopped, the excretion during the five days being similar to that obtained on a carbohydrate diet, and appreciably less than that observed in the preliminary period on the same days. The two rats whose behaviour is described were investigated together with three other pairs. In two of the other pairs the same augmentation of ketonuria was observed, while in the remaining pair there was no augmentation. The smallest dose of insulin following which this effect has been observed was a dose of 0.8 units per day, given in four injections of 0.2 units each to rats weighing 180 and 190 grm. This dosage produced the augmentation without the accompaniment of hypoglycæmic symptoms.

A second response to the injection of insulin is shown in Fig. 2, which illustrates an experiment carried out in December. In this experiment, as in the other, the rats were given a fat diet for a preliminary period in order to determine the normal ketonuria. This is shown as before as a broken line, and was less in magnitude than the normal

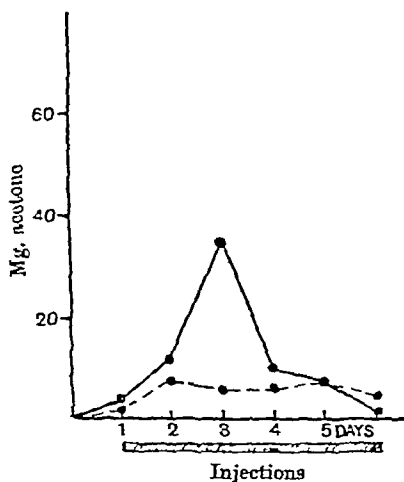


Fig. 2. Showing augmentation of ketonuria by insulin on second day of injections only. No augmentation on 1st, 3rd, 4th and 5th days of injections. Experiment carried out in December. See text.

September ketonuria. After an interval of 21 days a second period began, and injections of insulin were given from the second to the sixth days of the fat diet. The augmentation of the ketonuria was observed on the third day only. The dosage employed was 0.7 units per rat per day (the rats weighed 160 and 170 grm.) given in three doses of 0.2 units, and one dose of 0.1 unit. A similar experiment on a second pair of rats carried out at the same time gave a precisely similar result. Experiments carried

out in late October gave results resembling the September result in that the augmentation was maintained for two days or, when the dose of insulin was as large as 1.2 units a day, even for three days; but resembling the December results in that the lower doses of insulin produced no augmentation on the first day of injection, while on the fourth day of injection, the ketonuria was no greater than in the preliminary experiment.

The third type of response to the injection of insulin is shown in Fig. 3, which records an experiment carried out in June. There was no

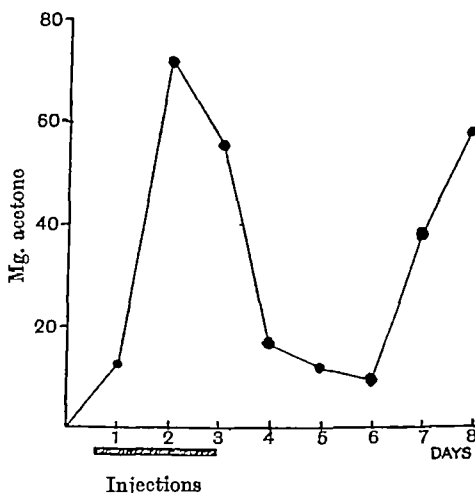


Fig. 3. Showing the second ketonuria occurring in summer about the 6th-8th days of fat diet when injections of insulin are given on the 2nd and 3rd days.

preliminary period of fat feeding, and it is impossible to say whether the large ketonuria observed on the two days of injections was larger than it would have been without the injections. In June and July the average ketonuria on the first day of insulin injections (being the second day of fat diet) was in five experiments 57 mg. (reckoned as acetone). The average ketonuria in the absence of injections was on this day 35 mg., taking this average from seven experiments done during the same weeks as the insulin experiments. This difference is scarcely significant. It may well be that insulin does not augment the already large summer ketonuria.

On the other hand, while the ketonuria was always high on the first day of injections, on the second day of injections in four out of five experiments it fell, at a time when in the absence of insulin it would have

been rising. This fall in one experiment was such as to mean almost a disappearance of ketonuria.

The main interest of the effect of insulin in June and July lay, however, not in its immediate, but in its late effect. When the injections were stopped (see Fig. 3) the ketonuria declined on the fourth, fifth and sixth days, and then again rose on the seventh and eighth days. This curious second rise in the ketonuria occurred in each of the five experiments in which the ketonuria was examined so long after injection.

### *The relation of ketonuria to hypoglycæmia.*

In several experiments in which the augmentor effect of insulin on ketonuria was observed, it was seen that the doses of insulin used produced no obvious symptoms. The most useful information about the level of the blood sugar was obtained from some experiments carried out at the beginning of November. A group of ten rats of similar weight were given a fat diet for six days. The excretion of acetone bodies was determined in two pairs, and the remaining six rats were killed at different stages of the experiment to determine the blood sugar. Injections of insulin, each 0.2 units, were given to each rat four times a day. The blood sugar was determined one hour after the last injection. The rat killed at the end of the first day of injections had a blood sugar of 0.073 p.c. On this day the insulin produced no augmentation of ketonuria. At the end of the second day two rats were killed, the blood sugars being 0.035 and 0.055 p.c. One rat killed on the third day had a blood sugar of 0.050 p.c. On the second and third days of insulin, an augmented ketonuria was observed. On the fourth and fifth days the blood sugar percentages were both 0.060, and on these days there was no appreciable ketonuria, the excretion being less than in the control period. While it appeared that the greatest ketonuria was associated with the lowest blood sugar value, there was no reflection in the blood sugar figures of the striking change from the high ketonuria of the third to the negligible excretion of the fourth day.

### *The liver glycogen.*

The unexpected effect of insulin in increasing ketonuria called to mind its unexpected effect in diminishing liver glycogen first demonstrated by Dudley and Marrian(10). It seemed probable that there was some connection between the two phenomena. To investigate this it was first necessary to determine the changes which took place in the

liver glycogen when rats were fed on a fat diet without insulin injections. These proved to be of considerable interest.

The first observations were made in October. Fifteen male rats, each weighing about 120 gm., were given a fat diet. Three were killed at 24 hour intervals for the determination of liver glycogen. The pieces of liver were transferred to tubes containing hot potash, and the tubes immersed in a boiling water bath within one minute of crushing the skull of the rat. We are indebted to Prof. Lovatt Evans for advice on the method of estimating the glycogen. The results are given in Table III.

TABLE III.

Each figure is the percentage of liver glycogen present in one rat, killed after it had been given a fat diet for the time shown.

	24 hours	48 hours	72 hours	96 hours	120 hours
	0.035	0.37	0.5	0.62	1.48
	0.11	0.41	0.75	1.80	1.51
	0.12	0.555	1.95	2.28	1.68
Average	0.09	0.44	1.06	1.57	1.56

The last line of Table III gives the average of the glycogen percentages, which is seen to rise steadily during the first four days to the

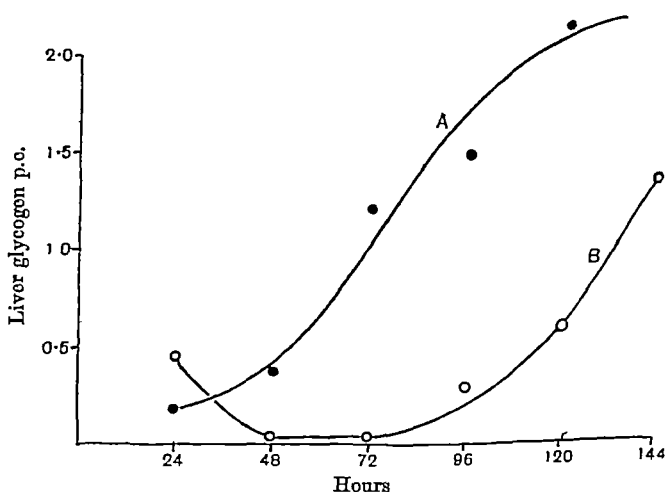


Fig. 4. Curve A shows rise of liver glycogen percentage in rats given a fat diet in November. Each point is the average value of three rats. Curve B is the record of a similar experiment in which 0.8 units insulin were injected into each rat daily from the second day of the fat diet to the end of the experiment. The curve shows the initial inhibition of the rise by the insulin. The inhibiting action begins to fail on the third day of injections.

appreciable amount of 1.5 p.c., from an initial figure of less than 0.1 p.c. The results of a similar experiment carried out at the beginning of November are shown in Curve *A* of Fig. 4. in which the figure for the rats killed at 120 hours was 2.2 p.c. Two other experiments in November gave similar results. The increase in the glycogen is reminiscent of, though more considerable than, the rise in liver glycogen observed by Barbour, Chaikoff, Macleod and Orr(11) in rats kept without food; they found a percentage of 0.16 at the end of 24 hours, which rose to 0.32 at the end of 48 hours.

A further point of interest lay in the values for the liver glycogen determined at the end of 24 hours of the fat diet. These became higher towards the end of November than they were at the beginning of October. In Table IV are given the results for the individual rats at different times.

TABLE IV.

Percentage of liver glycogen in rats killed after 24 hours' fat diet.

	11 Oct.	1 Nov.	21 Nov.	30 Nov.	12 Dec.	2 Jan.
	0.035	0.03	0.08	0.5	0.12	0.24
	0.11	0.12	0.51	0.5	0.22	0.46
	0.12	0.425	0.57	0.86	0.86	0.63
Average	0.09	0.225	0.39	0.62	0.4	0.44

Taken together with the fact of the gradual decline of the ketonuria from summer to winter, the figures suggest that the liver glycogen after 24 hours of a fat diet is lower in October than in December. The few figures given in Table IV of course do not establish this point.

#### *Origin of the new liver glycogen.*

We have as yet been unable to make an exhaustive study of the origin of this liver glycogen, but some observations may be briefly described. From simultaneous determinations of the muscle glycogen, it did not appear that a simple transference of glycogen from the muscles was sufficient to account for the increase in the liver. Table V shows the figures obtained for muscle glycogen in two experiments. Each figure is the average of the glycogen percentages present in the right gastrocnemius muscles of three rats.

TABLE V.

Muscle glycogen during fat diet.

	24 hours	48 hours	72 hours	96 hours	120 hours
Exp. 1	0.25	0.25	0.14	0.16	0.2
Exp. 2	0.23	0.20	0.15	0.18	—

In each of the experiments the final is 0.05 p.c. lower than the initial figure. For a rat of 100 grm., having 50 grm. muscle, this fall represents a disappearance of 25 mg. glycogen. If this were transported to the liver, of weight 5 grm., it would cause a rise of 0.5 p.c. The rise observed in the first of these experiments was 1.87 p.c. (from 0.4 to 2.27) and in the second 1.1 p.c. Hence on the basis of these figures it does not appear that the rise in the liver is due to a fall in the muscle stores.

Similarly the rise does not appear to be due to a formation of glycogen from protein, for observations of the excretion of total nitrogen showed no rise. In Table VI are given the figures for the mg. nitrogen excreted by each of four pairs of rats given a diet of fat and carbohydrate for two days (in order to eliminate the effect of omitting protein) and then given a diet of fat only for four days. The determinations were made by means of Pregl's micro-Kjeldahl apparatus.

TABLE VI.

Diet 1st and 2nd days—75 p.c. rice starch, 25 p.c. filtered butter with salt mixture. Diet 3rd, 4th, 5th and 6th days—filtered butter with salt mixture. Figures are mg. total nitrogen.

Rats	1st day	2nd day	3rd day	4th day	5th day	6th day
1st pair	230	157	191	177	144	138
2nd „	324	162	182	113	206	83
3rd „	174	195	132	153	179	86
4th „	235	224	176	163	120	112
Average	241	184	170	151	162	105

The figures do not show that the protein metabolism of the rats was regularly stimulated by taking them, in January, from a diet of fat and carbohydrate to one of fat alone. There was, on the whole, a gradual fall in the total nitrogen excreted.

We think it probable therefore that the rise in liver glycogen is a new formation from fat, but our evidence does not prove this.

### *The action of insulin on liver glycogen.*

We were now in a position to investigate the action of insulin on the rise of liver glycogen described. Our observations were made in December and January at a time when the effect of insulin on ketonuria was of the kind shown in Fig. 2, in which the augmentation was only witnessed on the second day, or the second and third, of the insulin injections. The injections were given exactly as in the observations on ketonuria, at 10 a.m., noon, 2 p.m. and 4 p.m. daily, and each rat received 0.2 units at each injection. The rats for glycogen determinations were killed one

hour after the last injection. The results of one experiment are given in Table VII, together with the figures for the muscle glycogen.

TABLE VII.

Each figure is the mean percentage of glycogen in two rats. No determinations were made after 1st day of insulin injections, which was 2nd day of fat diet. Days are days of fat diet.

	3rd day	4th day	5th day	6th day
Liver	0.31	0.19	0.62	1.13
Muscle	0.30	0.41	0.34	0.31

The table shows that just as insulin injections cause an augmentation of ketonuria, which ceases after one or two days, so they cause an inhibition of the rise in liver glycogen, which similarly ceases after one or two days.

On the third and fourth days of fat diet, the liver glycogen figures, under the influence of insulin injections, were 0.3 and 0.19 p.c. respectively, whereas in the absence of these injections the figures were about 1.0 and 1.5 p.c. On the other hand, by the fifth and sixth days, the liver glycogen rose in spite of the continuance of the injections. The relation of the rise to that occurring without insulin injections is clearly seen in Fig. 4, in which the values from another insulin experiment are plotted in Curve B. At the end of 24 hours' fat diet without insulin the glycogen was 0.45 p.c. Injections on the next two days reduced the glycogen to a negligible quantity, but thereafter failed to prevent a rise to 1.4 p.c. on the fifth day.

In the experiment in Table VII the figures for muscle glycogen are higher than those which were obtained without insulin injections.

#### DISCUSSION.

The experiments described in the first part of the paper, demonstrating that, in rats eating a diet of fat, insulin increases the ketonuria, appeared at first to add one further complexity to the already sufficiently puzzling phenomena of metabolism. The experiments in the second part, which demonstrate that this effect occurs only in the condition in which insulin is able to reduce the liver glycogen to a low value, show however that there is no new difficulty. The effect on ketonuria might have been foretold from the previously known facts (a) that insulin reduces liver glycogen, and (b) that the amount of ketonuria is inversely proportional to the amount of liver glycogen. The problem of reconciling the anti-ketogenic action of insulin in the diabetic patient with the ketogenic action in the normal animal on a fat diet, is therefore essentially the



same as that of reconciling the glycogen-forming power of insulin in the diabetic with the reduction of liver glycogen seen in the normal animal.

The observations on the rise of glycogen in the liver of the fat-fed rat are of interest, as they throw further light on the cause of the diminution of liver glycogen by insulin. Burn and Marks(12) have shown that during the recovery from an insulin hypoglycæmia there is certainly a discharge of the existing glycogen stores, and that, when these are exhausted by thyroid feeding, no recovery is possible. But it has always seemed probable that the fall in glycogen was only in part due to this discharge. Many observers (Laufberger(13), Lesser(14), Cramer(15), Best, Dale, Hoet and Marks(16)) have expressed the view that one of the functions of insulin is to inhibit the new formation of glycogen. The evidence presented here is a demonstration that insulin has this property. In rats eating a fat diet there is a rise of liver glycogen, which is a new formation of glycogen, and which is inhibited by injections of insulin for two or three days; the duration of the inhibition depends on the dose employed.

The evidence presented on the origin of the glycogen is incomplete in quantity, but is a further instance of the appearance of carbohydrate in circumstances in which a transformation from fat is the only simple explanation. The figures of nitrogen excretion show that the removal of carbohydrate from the diet does not lead to stimulation of nitrogen excretion in albino rats during the winter, and there is no considerable change in muscle glycogen. In any event it is now accepted by most observers that muscle glycogen cannot act as a source of liver glycogen.

The observations which have been made on ketonuria in the rat by Wigglesworth(3), Levine and Smith(17), Anderson and Anderson(7), together with those here described, make it clear that in this animal Shaffer's views of the ketogenic and anti-ketogenic action of different food materials(18) have no application. Levine and Smith have calculated for one experiment that an observed excretion of 20 mg. acetone bodies should have been, on Shaffer's view, an excretion of 1592 mg.

Finally the seasonal variation in the excretion of acetone bodies by the rat affords matter for speculation. The rats used in our experiments were originally Norwegian rats. It may be supposed that, in their evolution, they have developed a mechanism to enable them to withstand successfully long periods of winter starvation, in which their energy is maintained by combustion of their fat stores. When food becomes plentiful in summer, the mechanism is no longer needed and is in abeyance.

# SUMMARY.

1. There is a great seasonal variation in the excretion of acetone bodies by rats fed with a diet of fat; the excretion is high in summer and low in winter.

2. Injections of insulin augment the small ketonuria observed in winter. The augmentation may not be seen until the second day of insulin injections, and it usually disappears by the fourth day of injections.

If insulin injections are given in summer, and then withheld, a second augmentation of ketonuria supervenes after a few days.

3. Experiments in winter months have revealed a steady rise in liver glycogen in rats on a fat diet, beginning at 0.1-0.4 p.c. on the first day, and rising to 1.5-2.0 p.c. on the fourth and fifth day. This rise is not accompanied by a compensating fall in muscle glycogen, nor by a rise in the excretion of total nitrogen in the urine.

4. Injections of insulin delay this rise in liver glycogen for one or two days. The delay in the rise of liver glycogen corresponds in time to the period in which insulin augments ketonuria.

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# CONCERNING THE "CENTRAL" CONTROL OF THE PERIPHERAL CIRCULATION.

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ANREP and STARLING(1) demonstrated that in the dog a reciprocal action existed between the blood-pressure in the head and neck and in the periphery; a rise in the former causing a fall in the latter and vice versa. These results they attributed to a direct influence of the vascular pressure on the vaso-motor and vagus centres. Hering(2) has described in detail a depressor reflex from the sinus caroticus, and in his recent monograph attributed the results of Anrep and Starling to the presence of this reflex in their preparations, although he did not offer any adequate proof of this contention. Anrep and Segall(3) in further experiments with the innervated heart lung preparation asserted that, even after complete excision of the sinus caroticus itself, a "central" action upon heart rate still existed, and Nash(4), using the same preparation, found that a rise in the cerebral blood-pressure still determined a fall of blood-pressure in the body.

During some experiments upon the cerebral blood vessels we obtained results which indicated that in the intact animal there was no reciprocal action between the blood-pressure in the circle of Willis and the peripheral blood-pressure, and in view of the divergence of opinion between the authors stated we extended our observations to elucidate this problem. In the experiments to be described, we have changed the blood-pressure in the circle of Willis by occluding the vertebral arteries. The occlusion of these arteries, while lowering the pressure in the circle of Willis, has no mechanical influence upon the general arterial pressure, and any reciprocal action between the pressure in the circle of Willis and the peripheral blood-pressure can be studied without the interference of the depressor reflex from the sinus<sup>3</sup>. In some experiments the carotid

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<sup>2</sup> Working on behalf of the Medical Research Council.

<sup>3</sup> Siciliano(5) mentions that he occluded the vertebral arteries, and found no noticeable effect on blood-pressure or heart rate.

sinus on the recording side has been denervated. In others both have been carefully stripped and cocainised, but results after this procedure did not differ from those with the sinus intact.

### *Experiments upon rabbits.*

The rabbit was first chosen for experiment because in this animal it is relatively easy to dissect both right and left vertebral arteries and to place loops around them from the neck.

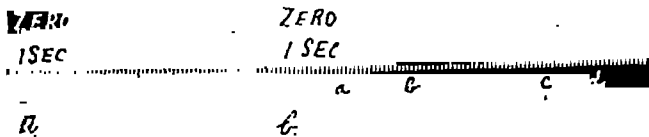
In the course of the experiments, which were made under urethane anaesthesia(c), it became apparent that this drug gave a preparation from which depressor responses to various stimuli were very easily obtained. The observations on the rabbit therefore have been supplemented by some made on dogs and cats using different anaesthetics.

The vertebral arteries of the rabbit were isolated in the following manner. Each subclavian artery was exposed through an incision at the root of the neck and ligated. The ligature thread was then utilised to pull upon the artery, new threads being tied nearer the heart as dissection of the artery proceeded. In this way the vertebral artery was drawn into view and a thread looped around it. Traction on the subclavian artery was then released. By drawing on the thread round the vertebral artery the blood flow through it could be stopped. The left vertebral artery, being placed deeply in the thorax, was the more difficult to dissect. In experiments in which the circle of Willis pressure was measured a cannula facing headwards, was placed in the left common carotid artery, all branches except the internal carotid being tied off. The left sinus caroticus was carefully stripped and 20 p.c. novocain applied. The peripheral pressure was measured from the left femoral artery in all cases.

That occlusion of the vertebral arteries causes a fall in the circle of Willis pressure could be easily shown in such a preparation.

In Fig. 1, (a) is shown the effect on the femoral artery pressure of occluding the right and left vertebral arteries simultaneously. There is no alteration in the peripheral pressure. The rise of pressure from occluding both common carotids is shown in (b), the vertebrals being patent. (c) Shows the effects of occluding the right carotid. This gives a rise of peripheral pressure. Both vertebral arteries are then occluded, but this does not produce a further rise of peripheral pressure. (d) Shows the effects on the peripheral pressure of occluding first both carotids and then both vertebrals. In the rabbit this results in a further asphyxial rise and marked slowing of the heart.

mm Hg.



50 mm. Hg.

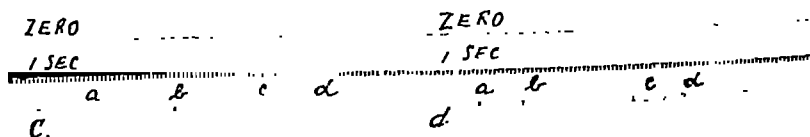


Fig. 1. Rabbit. Urethane.

(a)  
Right and left vertebral arteries  
simultaneously occluded.

(c)  
a. Occlusion of right common carotid.  
b. Simultaneous occlusion of both vertebrals.  
c. Release of both vertebrals.  
d. Release of right common carotid.

(b)  
a. Occlusion of right common carotid.  
b. Occlusion of left common carotid.  
c. Release of left common carotid.  
d. Release of right common carotid.

(d)  
a. Occlusion of left common carotid.  
b. Occlusion of right common carotid.  
c. Occlusion of both vertebrals.  
d. Release of all vessels.

In a series of similar experiments a rise of peripheral pressure has never followed a fall of circle pressure, as would be required if a central control was in operation. In some cases, as already stated (6) a fall of pressure has been produced by traction on the vertebral arteries, but this has been explained in the article already referred to (6).

The reverse experiment of increasing the pressure in the circle of Willis is not accompanied by such clear cut results. Fig. 2 illustrates

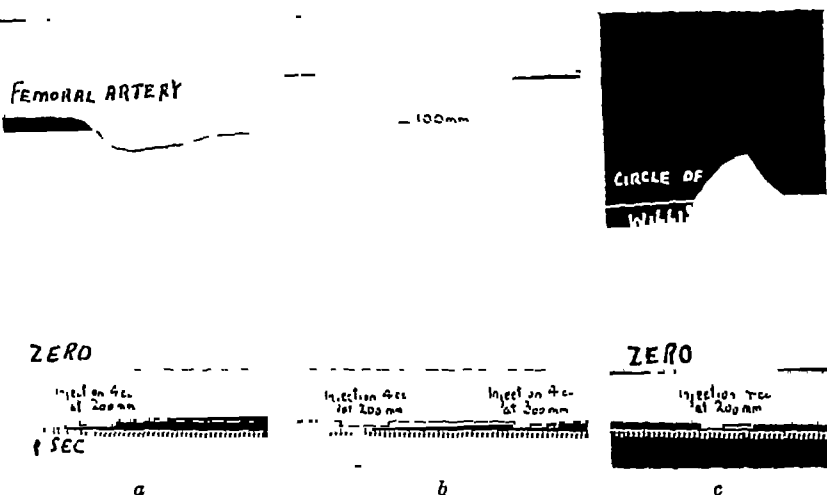


Fig. 2. Rabbit. Urethane.

(a) Effect on general blood-pressure of injection of 4 c.c. of defibrinated blood headwards into the common carotid, the external carotid being tied. Time of injection  $7\frac{1}{2}$  sec. Pressure of injection 200 mm. Hg.

(b) Injection of 4 c.c. of defibrinated blood in 10 sec. at 200 mm. Hg. after denervation of sinus caroticus, also 4 c.c. in  $7\frac{1}{2}$  sec. at 300 mm. Hg.

(c) Circle of Willis pressure. Shows increase of pressure from injection of 4 c.c. blood at 200 mm. Hg. pressure in 10 sec.

an experiment in which defibrinated blood at  $37^{\circ}$  C. was forced into the right internal carotid artery after complete denervation of the sinus. There was no effect on the peripheral blood-pressure. A tracing from the left internal carotid of the circle of Willis pressure was then taken from which it was seen that equal amounts of blood injected at the same rate produced considerable rises in circle pressure. In other experiments however the effect of the added volume of blood was manifest. This will be discussed with the experiments on dogs.

A point of some interest with regard to the general rise of pressure from common carotid compression appeared in these experiments. In the rabbit it is immaterial where this vessel is compressed so far as the rise of pressure is concerned. The same degree and form of rise is produced by clamping the common carotid, the sinus caroticus, or the external carotid. The external carotid branches can be clamped as high as 1 cm. above the exit of the internal carotid artery with the production of a rise as high as that produced by clamping the common carotid (Fig. 3). These results were not materially influenced by denervation of the sinus.

The possible explanations would appear to be that the rise of pressure is mechanical, due to a sudden increase of the peripheral resistance, or that a reflex similar to that from the carotid sinus has an afferent supply from branches of the external carotid.

From a consideration of the shapes of the curves—the sharp rise on clamping and sudden fall of pressure after release—we are inclined to believe that the former explanation is correct.

ZETO  
SIGNAL  
1 sec

Fig. 3. Rabbit. Urethane, shows effect on femoral artery pressure of tying the external carotid artery 1 cm. above the sinus caroticus.

### *Experiments upon the dog.*

In the dog, in the majority of the experiments, the vertebral arteries were exposed through a longitudinal slit in the anterior end of the sternum. Loops were placed around them close to their origin from the right and left subclavian arteries. The threads were passed through small holes in a metal device held rigidly close to the vertebral arteries so that traction on the threads occluded these but did not materially displace the subclavian vessels. In some experiments the vertebrals were reached from the neck, the same procedure being adopted as in the rabbit. This method, though the more difficult, had the advantage of leaving an animal with a higher blood-pressure and a better vagal tone than when the thorax was opened. The general blood-pressure and the pressure in the circle of Willis were recorded from the femoral and external carotid arteries respectively in the same manner as described for the experiments

on the rabbit. Various anæsthetics have been employed, namely, morphia and chloralose, chloralose, morphia and paraldehyde, without influencing the results.

*The blood supply to the circle of Willis.*

In every experiment in which we have tested it, we have found that occlusion of the external carotid produced a greater fall in the circle pressure than occlusion of the internal carotid; in point of fact, occlusion of the latter produced only a slight or no fall in circle pressure. The external carotid anastomoses with the circle of Willis by means of the middle meningeal and ophthalmic branches from the internal maxillary artery; and it is evident that this anastomosis, which is very free, carries a definite amount of blood to the circle. In the cat, the anastomosis is anatomically much more extensive, and this, coupled with the fact that the internal carotid is very small, would indicate that the external carotid in this animal is an even more important channel of blood supply. The occlusion of one vertebral alone, it was immaterial which, often produced little or no change in the circle pressure; but the occlusion of both vertebrales simultaneously always lowered that pressure, though the amount varied considerably in different animals. If during this double occlusion, the common carotid was compressed, a further fall of circle pressure occurred, which was compensated to a varying degree by the rise in general blood-pressure which always occurred when the sinus was intact. No signs of asphyxia were ever seen during this procedure.

It is evident that the circle of Willis in the dog is supplied by blood by so many and generous channels that it is impossible to predict the result of the occlusion of any one of its afferent vessels.

*Influence of lowering the circle pressure upon the general blood-pressure.*

In agreement with the results obtained from the rabbit, we have consistently found that lowering the pressure in the circle of Willis by occlusion of both vertebral arteries has no influence upon the general blood-pressure. This has been found both in animals with high and low blood-pressures, and in those with good or poor vagal tone. The amount by which the pressure has been lowered has varied in different animals, amounting to about 50–60 mm. Hg in the extreme case, but usually to about 30–40 mm. Hg. In many experiments a lowered circle pressure has been maintained as long as 60 seconds and no alteration in general blood-pressure has been brought about by such a prolonged change,



Representative examples of these various occlusions are shown in Figs. 4 and 5.



Fig. 4.

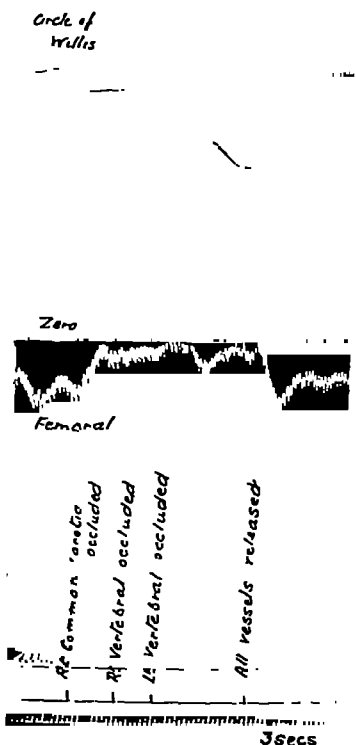


Fig. 5.

Fig. 4.  $\times \frac{1}{2}$ . Dog, chloralose. Effect of occlusion of the left vertebral artery upon the blood-pressure in the circle of Willis. The blood-pressure in the femoral artery remains unchanged. Prior to the observation, the right vertebral artery was tied off.

Fig. 5.  $\times \frac{1}{2}$ . Dog, chloralose. Effect of occlusion of right common carotid artery, right and left vertebral artery successively upon the blood-pressure in the circle of Willis. The blood-pressure in the femoral artery after the initial rise due to the occlusion of the right common carotid shows no further change.

*Influence of lowering the circle pressure upon the heart rate.*

Experiments have been performed especially to test this point, the vertebrals being reached from the neck, as in the experiments upon the rabbit.

No evidence has been obtained that a central control of heart rate exists; the lowering of circle pressure producing neither change in heart

rate nor in general blood-pressure. This result has been obtained both in dogs with fair or good vagal tone, and with circle pressure changes of varying degree and duration. Fig. 6 is a representative example. In this

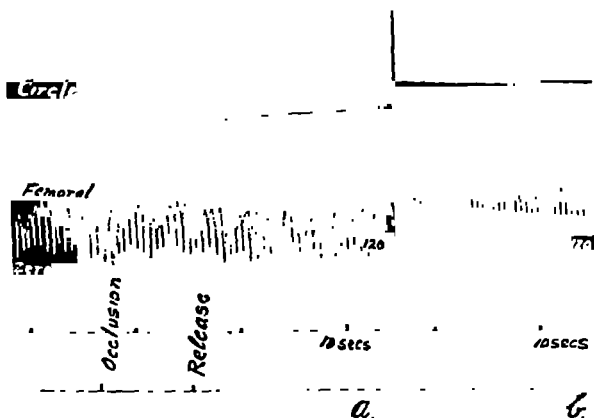


Fig. 6.  $\times \frac{1}{2}$ . Dog, chloralose. (a) Effect of occlusion of right and left vertebral arteries simultaneously upon the blood-pressure in the circle of Willis. No change in heart rate is recorded. Immediately after this observation the right vagus nerve was severed in the neck. The increased heart rate is shown in (b). Prior to this observation the right internal carotid artery was tied off.

animal the heart rate prior to occluding the vertebral artery was 96 per minute, during occlusion 96 per minute; no change occurring. Immediately after this observation the right vagus nerve was cut and the heart rate rose to 114 per minute. The vertebral artery was again occluded without any effect upon the heart rate or general blood-pressure, and immediately after the remaining vagus nerve was cut and the heart rate rose to 180 per minute.

#### *Experiments upon cats.*

The minute size of the internal carotid artery in the cat makes observations upon the circle pressure so hazardous that they were not attempted. Nevertheless, the experiment of vertebral occlusion was performed in three cats. In two of them the vertebral arteries were reached through a slit in the sternum, in the other they were isolated from the neck; this fact being confirmed post mortem. No alteration of peripheral pressure accompanied simultaneous occlusion of both vertebral arteries.

## DISCUSSION.

The conclusions arrived at from the foregoing experiments are drawn almost exclusively from the results of lowering of pressure in the circle of Willis and brain centres. An attempt was made to raise the circle pressure in dogs by injection of blood at high pressure through the internal carotid artery. In intact dogs this was found to be impracticable as the sudden increase of the venous inflow to the right heart resulted in first a slight fall and then a rise of pressure, exactly similar curves being produced by injection of blood into the saphenous vein. Nevertheless as very considerable alterations in circle pressure were produced by vertebral artery occlusion without effect on the heart rate or peripheral blood-pressure we feel justified in concluding that under physiological conditions, there is no significant "central" control of blood-pressure.

In "central" control two distinct mechanisms have to be considered, one is direct action on the medullary centres—presumably by pressure transmitted from the lumen of the capillaries to the nerve cells of the centres. Pressure upon the centres can apparently stimulate them as is seen when the intracranial tension is raised by any means. But this may mean that the small vessels of the centres are collapsed by a greater external pressure in the surrounding tissue, with resulting asphyxia, and cannot be accepted as evidence that the cells of the centres are themselves stimulated by pressure changes of physiological magnitude. The other possible mechanism is an afferent nerve supply to the cerebral blood vessels which is stimulated by alteration of tension within them in the same manner as the carotid sinus. This hypothetical afferent supply would act reflexly on the medullary centres to produce changes in blood-pressure and heart rate. Our experiments show, however, that no "central" control exists so that the inference may be drawn that the nerves supplying the cerebral vessels do not subserve a depressor reflex.

## SUMMARY.

1. In the rabbit, dog and cat, lowering the blood-pressure by as much as 50–60 mm. Hg in the circle of Willis does not produce any change in the peripheral blood-pressure or in the heart rate, both remaining constant.

2. In the rabbit, the rise in general blood-pressure which occurs upon occluding the common carotid artery is due, in large measure, to the mechanical factor of cutting off a large channel.

3. In the dog, and presumably the cat, the external carotid artery carries a considerable blood supply to the circle of Willis.

4. The experiments preclude the assumption that the nerves of the cerebral vessels are of an afferent nature subserving a depressor reflex.

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## THE INTERNAL CONDUCTIVITY OF NON-IRRITABLE MUSCLE.

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IN 1921, Foster and Moyle<sup>(1)</sup> reported their discovery that isolated frog muscle, kept in an atmosphere rich in oxygen at a temperature of 0° for about a fortnight, lost the property of responding to stimulation by contracting. Even powerful induction shocks failed to evoke any response, and the current of action was absent; the demarcation current was much modified. Neither rigor nor opacity developed in the muscles, and it became evident that to demonstrate any chemical or physical difference between these non-irritable muscles and normal muscle was a matter of great difficulty. It was further found that a considerable degree of irritability could be restored to the muscles by immersing them in electrolyte solutions. Lactic acid did not accumulate, in muscles kept under these conditions, above the normal value of 0.02 p.c., provided that oxygen was present at a pressure equal to or greater than that of atmospheric oxygen. At room temperatures a higher pressure of oxygen is required to prevent the accumulation of lactic acid<sup>(2)</sup>. On the other hand, the non-irritable muscles developed the typical amount of lactic acid, up to 0.25 p.c., when strongly heated or frozen, or when minced or treated with toluene, etc. Edsall<sup>(3)</sup> investigated the inorganic and organic phosphorus content of non-irritable muscles, and could find no significant difference from the normal. Foster and Moyle<sup>(1)</sup> found, however, that non-irritable muscles decrease in weight in solutions in which normal muscles showed no change in weight; they concluded that either the non-irritable muscles had developed an increased permeability, or that the number of osmotically active particles had diminished, possibly as a result of increased adsorption. The writer, whose attention was directed to the subject by Prof. Sir F. G. Hopkins, decided to test this latter suggestion, on the assumption that the osmotically active particles involved were ions, by determining the internal conductivity of the tissue in the normal and non-irritable states.

## APPARATUS.

The majority of our methods for the determination of the internal conductivity of cells are due to Höber, and are summarised in his book (4). In the first place he determined the capacity of a suspension of red blood corpuscles by comparison with a standard (aqueous) capacity by Nernst's Wheatstone bridge method (5). Improved forms of this apparatus have been used for the investigation of the plasma membrane of red blood corpuscles (6, 7) or of the capacity of tissues (8), and Höber's results have been confirmed (9). Subsequently Höber introduced other methods somewhat different in principle; high-frequency oscillations are induced in a circuit consisting of capacity, self-inductance, and small resistance, and measurements are made of the amount of damping which takes place when in some part of the circuit salt solutions of known conductivity are replaced by the cells to be examined. The test solutions may be placed in a thin-walled glass vessel within the self-induction winding (10), or in a special conductivity cell in parallel to the winding (11) or introduced into the dielectric of a special trough-like condenser in parallel to the principal condenser of the circuit (11). This last method was the one used in these investigations. Philippson (12) has also determined the impedance of cells and tissues at very high frequencies, by simultaneous measurements, with a triode voltmeter, of the current and potential across an electrolytic cell.

Two of the principles involved call for special mention. In the first place, the method depends on the fact that the capacity of a condenser is increased when a conducting layer is introduced into the dielectric; for example, when in the trough-like condenser the system glass-air-glass or glass-water-glass is replaced by glass-salt solution-glass or glass-tissue-glass. This increase in the capacity alters the natural frequency of the circuit. In the second place, while the resistance of a tissue to the passage of an ordinary alternating current is due largely to the relative impermeability of the cell-membranes to ions, this effect becomes less marked with increased frequency, and is negligible with oscillations of the order of  $10^7$  cycles per second. The impedance of a circuit containing capacity and resistance decreases with increased frequency. Höber repeatedly justified this thesis by experiments, in which he showed that while the Kohlrausch conductivity of a suspension of blood corpuscles is roughly that of an NaCl solution of 0.02 p.c., it is increased about ten times if the cell-membranes are destroyed by saponin; on the other hand, the conductivity to high-frequency oscillations is the same before

and after hæmolysis, and roughly equivalent to that of 0.2 p.c. NaCl solution.

Höber generated high-frequency oscillations in a circuit in which a series of Leyden jars discharged across a spark-gap, and were supplied with a high-tension current (30,000 volts) by an accumulator-fed alternating current generator, through a transformer. The introduction of the triode valve as a generator of oscillations (by Meissner in 1914) permitted the writer to use a much simpler and better arrangement. The triode used was an ordinary wireless "power" valve, pattern L.S.5 (Marconi). This generates oscillations in the circuit composed of the self-induction winding  $L_1$ , consisting of 9 turns of wire, 6 cm. radius, 13 cm. length, totalling about 0.009 millihenry, the variable condenser  $C_2$  which bridges the winding, and the mica condenser  $C_1$  (0.0005 microfarad, Dubilier make). The high-tension is obtained from dry batteries (Siemens) giving 300 volts, and tapped into the winding from the positive pole, from the negative pole to the filament of the valve, the negative pole of the low-tension source, and the grid of the valve through the high resistance  $R_2$  (100,000 ohms, wire wound, Mullard make). The filament is heated by a current from a 6-volt accumulator, through a rheostat  $R_1$  (by W. G. Pye). The oscillating portion of the circuit was wired up with 18 s.w.g. solid copper wire.

The receiving circuit is entirely built up with this wire, and every care taken to perfect insulation and reduce resistance. A single turn  $W$  of radius 6 cm. provides inductive coupling with the generating circuit, and two turns of the same radius, 0.7 cm. apart, are used for self-induction, which over the whole circuit totals about 1200 cm. The condenser  $C_3$  is a variable plate condenser working in air, capacity 0.0005 microfarad, and fitted with fine-adjustment and vernier (Geco-phone make). The condenser  $C_1$  is a glass trough with brass plates apposed to its sides, exactly similar to that used by Höber; it measures  $60 \times 60 \times 6$  mm. externally. The vacuum thermo-junction (supplied by the Cambridge Scientific Instrument Co.) is an improvement on the thermo-detector used by Höber, in that the resistance to the alternating current is low (0.33 ohm) and the elements are enclosed, on Lebedew's principle, in an evacuated glass bulb. The instrument is calculated to develop a potential of 6 millivolts from the iron-constantan thermocouple when the heating current is 420 milliamperes. The potential developed is measured by a galvanometer (W. G. Pye), a moving-coil instrument with an internal resistance of 13 ohms, giving a deflection at 100 cm. range of 3 mm. per microvolt and 45 mm. per microampere.

The circuit connecting the thermocouple to the galvanometer is earthed.

The frequency of the oscillations generated is determined by the self-inductance  $L_1$  and the capacity of the condensers  $C_1$  and  $C_2$ . When the latter is set at a capacity of 0.00002 microfarad, the frequency is of the order of  $10^7$  cycles per second. The oscillating current amounts to some 50 milliamperes, according to the specification of the valve. The two windings connecting the circuits inductively may accordingly be set 20 cm. apart. The method used is to fill the trough-condenser with the solution or tissue to be examined, to set the variable condenser  $C_3$  at a known point, to switch on the L.T. and H.T. supplies and to read the maximum deflection attained by the galvanometer (usually in about 45 seconds). The current is then switched off, the galvanometer allowed to return to zero, and the condenser  $C_3$  set at another point before switching on again. In this way resonance curves for a series of NaCl solutions of known strength were obtained, by plotting galvanometer deflection against settings of the condenser  $C_3$ ; examples of these curves are given. It will be noted that as the conductivity of the introduced salt solutions increases, the curves change both horizontally and vertically. The horizontal change is directly due to the increased capacity of the trough-condenser, requiring a compensatory decrease in the setting of the variable condenser to produce resonance with the generating circuit; unfortunately, in the case of tissues, these horizontal changes are not sufficiently regular to be reliable. Further, the amplitude of the galvanometer deflection is increased owing to the diminished resistance factor of the impedance of the circuit as a whole; this effect gives more reproducible results, and is the only one considered.

It may be observed that Höber was unable to detect the former effect. The apparatus described is a considerable improvement upon his, not only in the matter of cost and convenience, but also because condenser discharges can only produce damped trains of waves, succeeding each other with a frequency determined by the a.c. supply to the condensers, whereas the triode furnishes continuous oscillations of even strength.

#### EXPERIMENTS.

After a series of tests had been carried out, using NaCl solutions of 0.05, 0.1, 0.15, 0.2, and 0.25 p.c., determinations were made of the internal conductivity of normal, non-irritable, and partially recovered muscles. The gastrocnemius of the frog was used throughout. The normal muscles were separated as cleanly as possible, and washed for



two hours in ice-cold isotonic cane-sugar solution, with continuous stirring and frequent changes. Urano<sup>(13)</sup> and Fahr<sup>(14)</sup> found that six

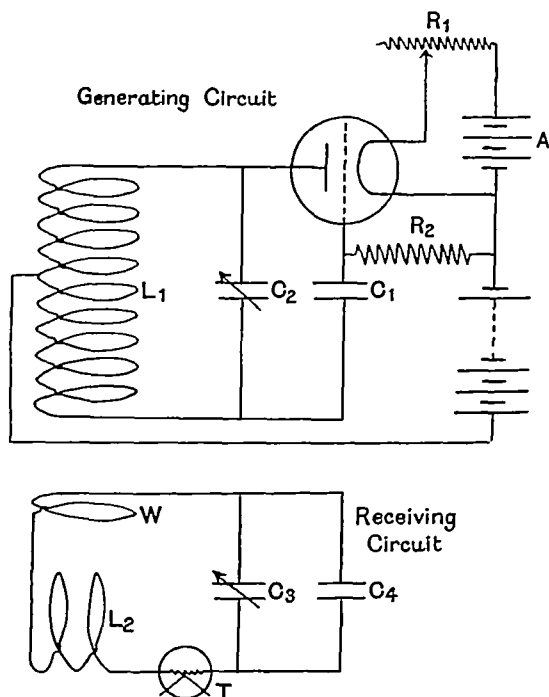


Fig. 1. Diagram of the apparatus. For explanation of the lettering see text.

hours' washing removed almost all the sodium from the frog sartorius, an indication that the blood and lymph had been completely removed. As it was thought undesirable to risk the effect of six hours' washing on the non-irritable muscles, this period was reduced; the ash analysis shows that the method was satisfactory. The muscles were then packed into the trough-condenser (whose dimensions are particularly suited for the purpose; about 35 muscles are required) as tightly as possible, with avoidance of air-bubbles and as little cane-sugar solution as possible. Undoubtedly the greatest experimental error comes in at this stage, and for this reason emphasis is not laid on the absolute values obtained. It is likely that the horizontal displacement of the resonance curves for muscle is due to errors in the packing process. The temperature of the trough-condenser is maintained at  $0^\circ$  throughout the experiments. The non-irritable muscles are treated in exactly the same way. After the determination, they were washed for one hour in isotonic

Ringer's solution at room temperature, till they showed signs of returning irritability. They were then washed again for two hours in ice-cold cane-sugar solution, and examined as before.

If the capacity changes are neglected, and the galvanometer deflections at the point of resonance alone considered, the results are as follows (the internal conductivities being expressed as equal to concentrations of NaCl of the same conductivity under the same conditions):

				% NaCl
Normal muscle	...	...	...	0.21
Non-irritable muscle	...	...	...	0.13
Partially recovered muscle	...	...	...	0.16

That no inorganic material has been lost from the tissue is shown by the following ash analyses, which also demonstrate the efficiency of the washing process:

State of muscle	Determined by	K %	Na %
Normal, fresh	Katz(15)	0.308	0.055
„ fresh	Fahr(14)	0.34	0.066
„ fresh	writer	0.34	0.059
„ washed 2 hours	writer	0.33	0.005
„ washed 6 hours	Fahr	0.32	0.002
Non-irritable	writer	0.33	0.058
„ washed 2 hours	writer	0.32	0.004

TABLE OF GALVANOMETER READINGS.

Condenser setting	NaCl solutions			Muscles		
	0.1 %	0.15 %	0.2 %	Non-irritable	Re-covered	Normal
26.0	—	—	20.8	—	—	10.9
27.0	14.2	29.4	41.3	22.3	25.0	22.7
28.0	19.6	39.6	53.5	30.4	37.6	45.6
28.5	26.0	41.0	52.8	34.9	41.7	53.1
28.75	27.4	—	—	—	—	—
29.0	31.2	40.1	50.8	37.7	43.1	55.4
29.25	30.9	—	—	37.9	—	—
29.5	31.8	37.6	—	36.9	41.5	53.8
29.75	31.3	—	—	—	—	50.7
30.0	31.0	31.4	31.9	34.2	37.8	47.1
30.25	29.1	—	—	—	—	—
30.5	28.2	26.6	—	—	—	—
30.75	—	—	—	—	30.0	31.5
31.0	23.4	—	20.7	26.0	—	26.4
32.0	16.3	—	12.5	—	—	—

The galvanometer readings are in cm. at 100 cm. distance, the sensitivity at this range 3 mm. per microvolt. The condenser settings are approximate percentages of the total capacity of 0.0005 microfarad.

## DISCUSSION.

Höber(11) found that while the Kohlrausch conductivity of normal frog muscle was equivalent to that of 0.02–0.04 p.c. NaCl, the internal

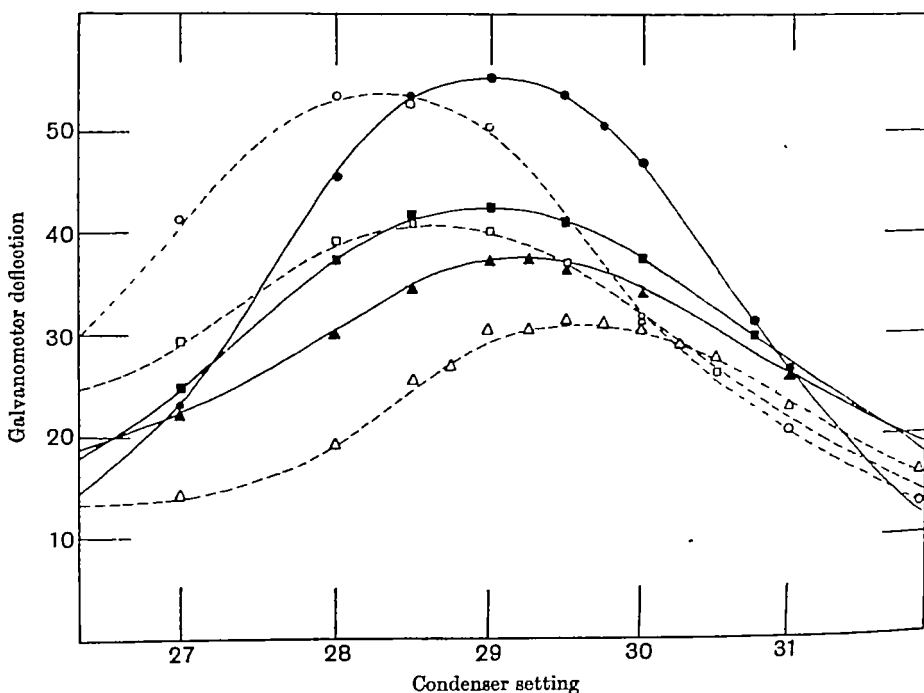


Fig. 2. Resonance curves obtained with salt solutions and tissues. The ordinates are galvanometer deflections in cm., the abscissæ settings of the variable condenser. The broken lines are resonance curves obtained with NaCl solutions: uppermost (circles) 0.2 p.c., middle (rectangles) 0.15 p.c., lowest (triangles) 0.1 p.c. The continuous lines are resonance curves from muscles: uppermost (circles) normal, middle (rectangles) partially recovered, and lowest (triangles) non-irritable.

conductivity corresponded to that of NaCl solutions of 0.22, 0.16, 0.14 and 0.2 p.c. Although these figures are greater than those directly read off, a correction for the dead spaces within and between the muscles having been applied, they are on the whole lower than the value (0.21 p.c.) given directly by the experiments described; on the other hand Philippson(12) obtained the still higher value of 0.31 p.c. It may be of interest to compare other measurements of the internal conductivity of cells. For mammalian blood corpuscles Höber(5, 10) obtained by various methods values ranging between 0.1 and 0.4 p.c. NaCl; Philipp-

son(12) found 0.15 p.c., and Fricke and Morse(9) 0.17 p.c. This last figure appears to be particularly reliable, having been obtained in the course of an elaborate investigation(16) of the capacity and resistance of disperse systems. Thornton(17), by studying the behaviour of suspended bacteria in an electric field, found their conductivities to vary over a wide range, but to be at times much greater than those indicated above. Brooks(18) obtained quantities of "pure" protoplasm from a slime-mould, and found its conductivity equal to that of 0.0085 p.c. NaCl, while Gelfan(19) obtained still lower values by introducing electrodes, with a micro-manipulation apparatus, into the protoplasm of various unicellular animals and plants and passing a direct current. In connection with these last experiments Kofoid has suggested a correlation between conductivity and activity of habit.

The most important point emerging from the experiments detailed in this paper is that the conductivity of non-irritable muscle is much less than that of normal muscle. This immediately suggests an explanation for the absence of irritability. Nernst(20) has propounded the theory, now very widely accepted, that electrical stimulation depends on alterations in the concentration of ions at membranes of restricted permeability within the tissues, and further that stimulation can only take place when these changes occur at more than a certain threshold rate and to more than a given threshold amount. It is therefore not inconceivable that in the non-irritable muscles the low conductivity prevents the concentration of the ions at any point being altered to the threshold extent, so that irritability is suppressed. At the same time, it remains possible that the loss of irritability is due to some change, perhaps of quite different nature, which has not yet been detected. The ash analysis of the non-irritable muscles washed in cane-sugar solution does not suggest that a marked increase in permeability to electrolytes has occurred. The osmotic experiments already quoted are evidence that the amount of water in the tissues has not diminished.

The question of the decreased conductivity of non-irritable muscle offers merely a wide field of speculation when the cause of the observed phenomenon is considered. Höber(11), noting that the internal conductivity of normal muscle was less than the amount of inorganic bases present and determinable by analysis might lead one to expect (ca. 0.64 p.c. NaCl), suggested that (a) ionic migration was retarded by viscosity, (b) ions were immobilised by adsorption or otherwise, or (c) a large proportion of the inorganic elements present, potassium in particular, was present in non-dissociating organic compounds. I am

indebted to Prof. A. V. Hill for suggesting that in considering these alternatives it must be remembered that 0.7 p.c. NaCl is approximately isotonic for frog muscle, *i.e.* that the internal conductivity is less than the osmotic properties, as well as the chemical composition, of the tissue would suggest. These difficulties are partly overcome on the hypothesis that a large proportion of the potassium is present in the ionic state, held by electrostatic attraction to protein anions in such a way as to be incapable of conducting an electric current, but remaining free to exert an osmotic effect. It might be anticipated that if the tissues contained a large quantity of immobilised ions, their capacity would be greater than that of electrolyte solutions of the same resistance; this is not realised in the resonance curves experimentally obtained, but it is doubtful if these curves have sufficient absolute accuracy to lend point to the objection:

There is at present no reason to suppose that the further diminution in conductivity in the non-irritable muscles is not a wholly distinct phenomenon, for which various explanations may be offered. Examination of the conditions under which irritability is lost and regained shows that too much emphasis is easily placed on the question of temperature. Since complete irritability may be restored to non-irritable muscle, without exposing them at any time to temperatures above  $0^{\circ}\text{C.}$  (21), the low temperature cannot be regarded as in itself significant in the loss of irritability. It seems preferable to regard the period in which irritability is lost as a period of *rest*, throughout which stimuli of whatever sort are absent; and it is interesting to compare experiments, such as those of Carey (21), in which hypersensitivity develops in muscles as a result of over-stimulation. In the absence of stimulation, it is not inconceivable that even at low temperatures anabolic processes tend to increase relatively to catabolic changes, so that an increase in the amount of non-dissociating compounds at the expense of electrolytes might take place. It may be pointed out, moreover, that during the period in which irritability is lost, non-volatile waste products must accumulate in the tissues; this suggests a simple interpretation of the process of recovery. The metabolism of the tissue, under such conditions, is, however, very small. It may be calculated, from the rate of accumulation of lactic acid in muscles in an atmosphere of nitrogen at  $0^{\circ}$  (1), that the output of  $\text{CO}_2$  by the muscles is approximately 0.18 mg. per gm. per day, on the assumption that only one-fifth of the lactic acid formed under aerobic conditions is oxidised. This is in good agreement with the figures obtained by Lascelles, which appears to justify the assumption. The criticisms

of the work of Foster and Moyle advanced by Bottazzi<sup>(22)</sup> are not relevant to the present discussion.

## SUMMARY.

1. An improved apparatus for the determination of the internal conductivity of tissues is described.
2. The conductivity of non-irritable muscle is less than 60 p.c. of that of normal muscle.
3. The bearing of this result on the problem of the loss of irritability is discussed.

I am much indebted to Prof. Sir Frederick Hopkins for his continued interest and encouragement; to Miss C. E. Lascelles for the preparation of the non-irritable muscle, and for advice; to Mr Ratcliffe for invaluable help in the designing of the oscillator; and to the laboratory assistants, H. Hall, F. Johnson, and W. Mowll, for their very practical assistance.

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# A NEW ELECTRICAL RECORDING SYSTEM FOR PHYSIOLOGICAL WORK.

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Cambridge*).

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THE recording of minute and rapid electrical changes such as occur in living tissues is in itself a difficult physical problem. Since the advent of valve amplifiers the smallness of these changes does not matter much, as they may easily be magnified many times; but their rapidity is still a great difficulty. No instrument has yet been designed which will give an exact record of the electric response accompanying a single impulse in one nerve fibre.

Several systems have been used to record these rapid electric changes; these are, the capillary electrometer, the string galvanometer and two forms of oscillograph—the cathode ray oscillograph used by Gasser and Erlanger<sup>(1)</sup> and the moving-loop oscillograph used by Rosenberg<sup>(2)</sup>. The string galvanometer is of great value in recording the comparatively slow electric changes accompanying the heart beat; in the ordinary pattern the inertia of the string prevents it from giving an accurate record of electrical changes as rapid as those of the action potential of nerve. The records can be corrected by a mathematical analysis<sup>(3)</sup>, but this is both laborious and very difficult<sup>(4)</sup>. Special types of string galvanometer have been made by Einthoven for recording very rapid changes, but some loss of sensitivity is involved when the period of the string is quickened, and unfortunately the string galvanometer does not readily lend itself to use in conjunction with valve amplifiers owing to the fragility of the string, which is easily broken by any chance disturbance affecting the amplifier.

The capillary electrometer in combination with a valve amplifier as used by Adrian<sup>(5)</sup> forms a convenient recording system; its sensitivity is great, and records obtained with it, though greatly distorted by the damping of the mercury, may be analysed with the machine invented by Keith Lucas. This analysis, however, depends on the setting of the cross wire of an eye-piece tangentially to the record, and this is often extremely difficult if the record is at all faint. The high degree of damping



of the mercury may also become a source of error, for when a train of sensory impulses is recorded with the capillary electrometer, the descending record of one action potential interferes with the ascending portion of the next, and if the impulses are following each other rapidly it is hard to count the number of impulses in the record.

In both the string galvanometer and the capillary electrometer the light is limited by having to pass through a microscope objective, so that a very accurate optical system must be used if the photographic surface moves much faster than one metre per second.

The moving loop oscillograph used by Rosenberg(2) is essentially similar to the Duddel oscillograph. Its periodic time is  $1/7000$  sec. The records of nerve action potentials obtained with it appear to be slightly distorted, as when the applied potential changes rapidly the loop over-swings. The chief disadvantage appears to be lack of sensitivity, for an amplification of about 10,000 is required to record the action current of a nerve trunk in response to maximal electrical stimulation. Much greater amplification would be necessary to record sensory impulses, and the construction of a stable amplifier of such power would be a formidable problem.

The cathode ray oscillograph is not open to any of the above objections as its moving system (a stream of electrons) has neither appreciable mass nor damping, and no part can be broken by excessive deflections; but it has a new and serious drawback, namely, that the stream of cathode rays has a very low actinic power, and a single excursion will not affect a photographic plate; in order to obtain a record some thousand excursions must be superimposed. At present this limits the application of the instrument to changes that can be exactly repeated many times, so that it is not available for recording changes which depend on any spontaneous activity of living tissues. In the hands of Gasser and Erlanger(1, 5) the cathode ray oscillograph has given valuable information about the time relations of the action current evoked by electrical stimulation of a nerve trunk, but at present it cannot be used to record discharges of impulses like those Adrian(4) has shown to ascend a nerve from sensory end organs, nor is it suitable for recording irregular discharges of impulses leaving the central nervous system.

On account of these disadvantages inherent in the existing recording systems, it seemed worth while to try to construct some new instrument which should be better suited to physiological work. With this object in view the moving-iron oscillograph, and an amplifier suited to work with it, were designed and made in the Physiological Laboratory at Cam-

bridge. Though at present it is only in its experimental stage, and can be much further improved, this instrument promises to be well suited to the recording of the wave of action potential in nerve. Some of its advantages are as follows:

It can give a record of action potentials in nerve which is accurate to 95 p.c. without analysis.

When used with its amplifier it can record the action potential accompanying the activity of a single fibre in a large nerve trunk, such as the sciatic of the frog.

It is practically unbreakable.

Its optical system is extremely simple; and as the light is not limited by having to pass through a microscope objective, records can easily be obtained on plates with an effective speed of 10 metres per second.

Its adjustments are easily made, and its sensitivity can be varied over a wide range by altering the number of valves employed.

#### THE MOVING-IRON OSCILLOGRAPH.

The principle of this instrument is as follows: a piece of iron situated in a magnetic field is acted on by a force, and when the field alters the force on the iron alters proportionally. The potential to be recorded is applied across the coils of an electromagnet, and the alteration produced in the field is registered by the movement of an iron tongue. The system is in fact the same as that used for moving the diaphragm of a telephone. Fig. 1 is a photograph of the present oscillograph.

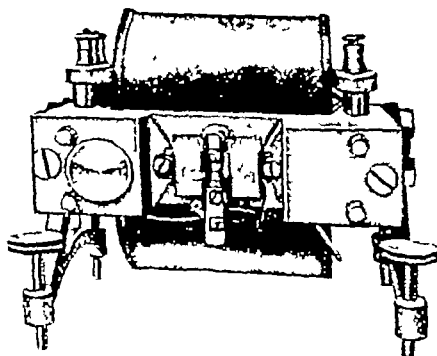


Fig. 1. The Oscillograph.  
*This instrument is the subject of patents*

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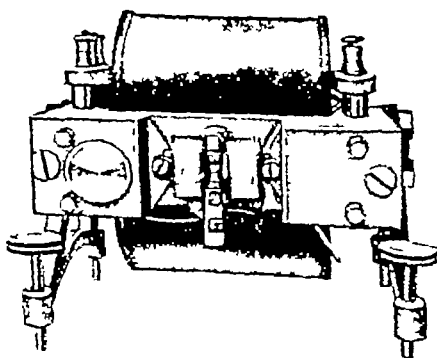


Fig 1. The Oscillograph.  
*This instrument is the subject of patents )*

Fig. 2 *A* gives a diagrammatic sketch of the apparatus. An electro-magnet produces a field, which is concentrated on to an iron tongue by

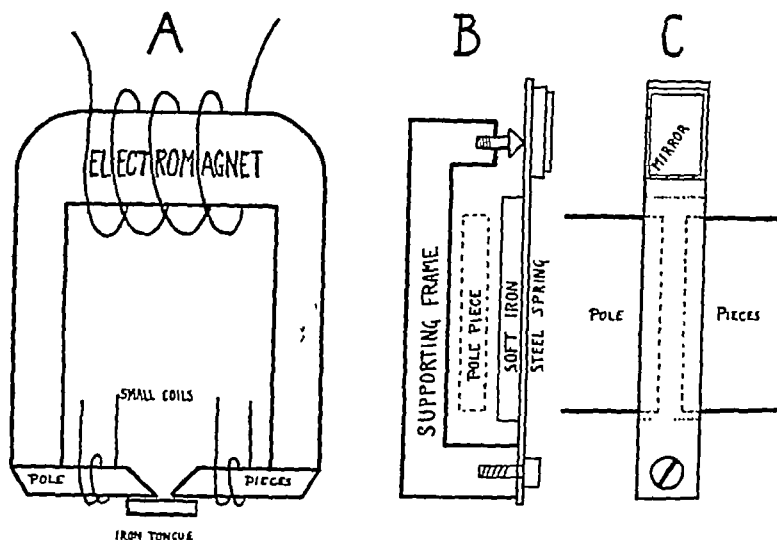


Fig. 2. *A*. Diagram of the oscillograph. *B*. Enlarged section of tongue. *C*. Enlarged plan of tongue.

laminated soft-iron pole pieces; these pole pieces carry small coils of many turns of fine wire.

Fig. 2 *B* is an enlarged section of the tongue and its supports. It is built up of a piece of clock spring, to which are riveted a piece of soft iron and a piece of steel, the latter carrying a small mirror. The tongue is clamped at one end and at the other rests on a screw centre; by this method of mounting, the movements of its centre are considerably magnified as they are transmitted to the mirror. The position of the frame is adjustable, and thus the gap between the tongue and the pole pieces can be varied. A constant current is passed through the large coil sufficient to magnetise the pole pieces to about half saturation, the tongue is therefore acted on by a force towards the pole pieces; any current in the small coils alters the force on the tongue, causing it to bend and deflect a spot of light reflected from the mirror.

The oscillograph in this simple form could not give at all an accurate record of potentials which vary rapidly; for owing to the self-inductance of the coils the current in them would not closely follow the applied potential changes; also because of its mass, the movement of the tongue would not exactly correspond with the changes in the magnetic field.

*Inductance of the Coils.* Fig. 3 *A* illustrates how instantaneous potential changes are distorted by the inductance of the coils. When

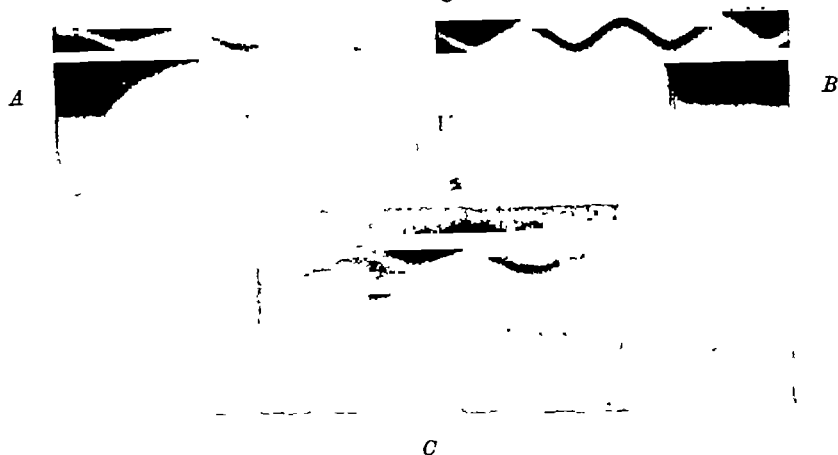


Fig. 3

*A* and *B*. Oscillograph records of instantaneous potential changes.

*A*. Distortion due to the inductance of the coils unbalanced by the non-inductive resistance of the valves.

*B*. Distortion due to the mass of the tongue without oil damping, the inductance of the coils compensated by a non-inductive resistance.

*C*. Record of condenser discharge, the potential falling to 5 p.c. in 0.0005 sec.; tongue undamped. There is no overswing.

Time marker 200 D.V. per sec. Read from left to right.

the potential falls the current in the coils and therefore the deflection of the iron tongue is sustained by the E.M.F. induced in the coils by the falling current, similarly when the potential rises the current is opposed by the back E.M.F. and only rises slowly. This distortion can be almost entirely eliminated if a large non-inductive resistance is included in the circuit in series with the small coils as in Fig. 4.

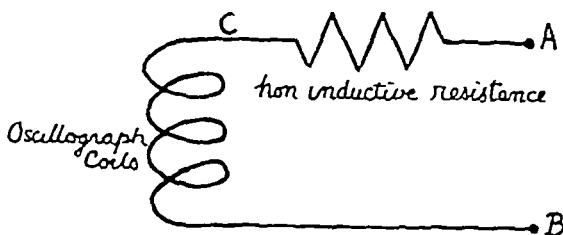


Fig. 4. Diagram of non-inductive resistance in series with the oscillograph.

If a potential is applied across  $AB$  (Fig. 4) after a short interval an equilibrium is reached with a steady current flowing in the circuit. The potential at  $C$  will be much lower than that at  $A$  owing to the presence of the non-inductive resistance.

During the interval before the current has reached its equilibrium value the effective resistance of  $CB$  is much greater than its steady value and the potential at  $C$  builds up towards that at  $A$ , and an E.M.F. much larger than the equilibrium E.M.F. across  $CB$  is available to overcome the back E.M.F. produced in the coils. Thus the current changes in the coils will follow much more nearly the potential changes occurring between  $A$  and  $B$ .

It will be seen that this is a direct corollary of the law of Helmholtz, which states that the current at any instant after closure of an inductive circuit is given by

$$I = \frac{E}{R} \left( 1 - e^{-\frac{Rt}{L}} \right),$$

where  $I$  = current  $t$  sec. after closure of circuit (amperes),

$E$  = E.M.F. (volts),

$R$  = resistance of circuit (ohms),

$L$  = self-inductance of circuit (henries).

Thus the percentage of the final current flowing at any instant depends on the ratio of  $L/R$  and, as was pointed out above,  $R$  can be increased by including a non-inductive resistance in the circuit; thus the current changes in the coils may be made to follow much more nearly the potential changes occurring between  $A$  and  $B$  (Fig. 4).

Fig. 3B is the record obtained by applying an instantaneous rise and fall of potential across the coils with a large non-inductive resistance in series with them. The current is here delayed very little but the record is not a true representation of the potential changes as it is still distorted by the oscillations of the tongue.

The internal resistance of a triode valve forms a suitable non-inductive resistance when the coils are connected in the anode circuit; if a potential change is impressed on the grid of the valve, the anode circuit current will change proportionally, and a large potential is available from the anode battery to overcome the back E.M.F. induced in the coils by the current change in them. By using valves of high internal resistance and coils of low self-inductance this distortion could be practically eliminated; but the self-inductance of the coils can only be reduced by decreasing the number of turns in them and this leads to a proportional

decrease of sensitivity. With valves of high impedance the plate current is very small, and the deflections are thus limited. In practice a compromise must be made between sensitivity and speed of deflection. It is possible by the use of a number of coils on the pole pieces, each connected in the anode circuit of a separate valve, to obtain less distortion at any sensitivity than would be possible with a single coil.

The present instrument has six coils, each of 3000 turns, but it has not been found worth while as yet to use more than two. Each of them is connected in the anode circuit of a valve having an internal resistance of about 6000 ohms.

*The moving system.* So far we have discussed the way in which the change in the magnetic field differs from a change of potential applied to the coils and it has been shown that the changes in field may be made to follow the potential changes very closely.

The moving system possesses mass and a certain degree of damping, and therefore cannot move instantaneously.

When the tongue is at rest in any position the two forces acting on it are in equilibrium and the force due to the field is equal to the restoring force of the elasticity of the tongue. When the field alters the tongue is driven to its new position by the difference between these forces. The motion of the tongue is best made clear by means of a diagram (Fig. 5). The tongue is at rest in a position *A*; now imagine

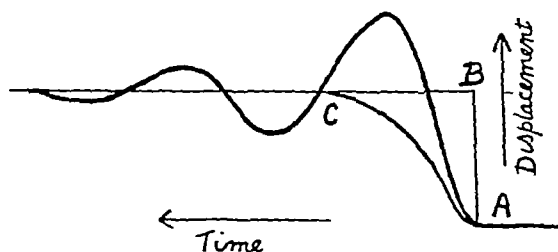


Fig. 5. Diagram of movement of the tongue when an instantaneous change occurs in the field.

the field to change instantaneously, the equilibrium position of the tongue is now at some point *B*, but the mass of the tongue prevents it from reaching this position instantaneously so it will start a damped vibration about *B*; it arrives at *B* after one quarter of its periodic time, but it arrives with a considerable velocity and continues vibrating about *B* until the energy of this vibration is absorbed by the damping. If the degree of damping is increased to the critical value this energy



is entirely absorbed in the first quarter vibration, but the motion of the tongue is slowed and it moves to the new position along a slope  $AC$ . The motion may be slowed still further if the damping exceeds the critical value.

In practice if a film of thin machine oil is introduced into the space between the tongue and the pole pieces the tongue will be fully damped, and when an instantaneous potential change is applied the full deflection is reached in about 0.0002 sec. with no trace of overswing (see Fig. 10  $A$ ).

Unless the potential changes vary very rapidly air damping is sufficient to prevent overswing. Fig. 3  $C$  is a record of a potential which rises to 95 p.c. of its full value in 0.0005 sec. and this is comparable with the rising phase of a nerve action potential at body temperature. Here the tongue shows no tendency to overswing.

Clearly the rate of movement of a tongue which is damped so that it just fails to overswing depends on its natural rate of vibration, and this has accordingly been made of a high order (5000 per sec.) by keeping the tongue as light and stiff as was practical, and by supporting it at both ends.

*Straight line relationship.* If the deflections of the tongue are to be proportional to the applied potentials certain conditions must be satisfied:

(a) the current in the coils must be proportional to the applied potential;

(b) the changes in the field must be proportional to the changes of current in the coils;

(c) the change of force of the tongue must be proportional to the field changes;

(d) the restoring force acting on the tongue must be proportional to the displacement.

(a) is satisfied, as Ohm's law applies here, (d) also is satisfied for the displacement of a member supported at both ends varies directly as the force acting on it, so that the restoring force is directly proportional to the displacement. In (b) and (c) slight deviations from direct proportion will result from the hysteresis of the iron of the pole pieces and of the tongue.

*Hysteresis.* The effect of the hysteresis is that when the pole pieces have been magnetised in either direction they will retain a small fraction of that magnetism after the magnetising field has ceased to act on them; this will mean that after a large deflection the base line is slightly shifted in the direction of that deflection. But the pole pieces are built up of

laminæ of pure soft iron which has been annealed and the hysteresis of such iron is very small.

If the current in the coils is plotted against the deflection for any cycle of magnetisation a hysteresis loop for the iron of the pole pieces and tongue is obtained. Fig. 6 shows two such loops.

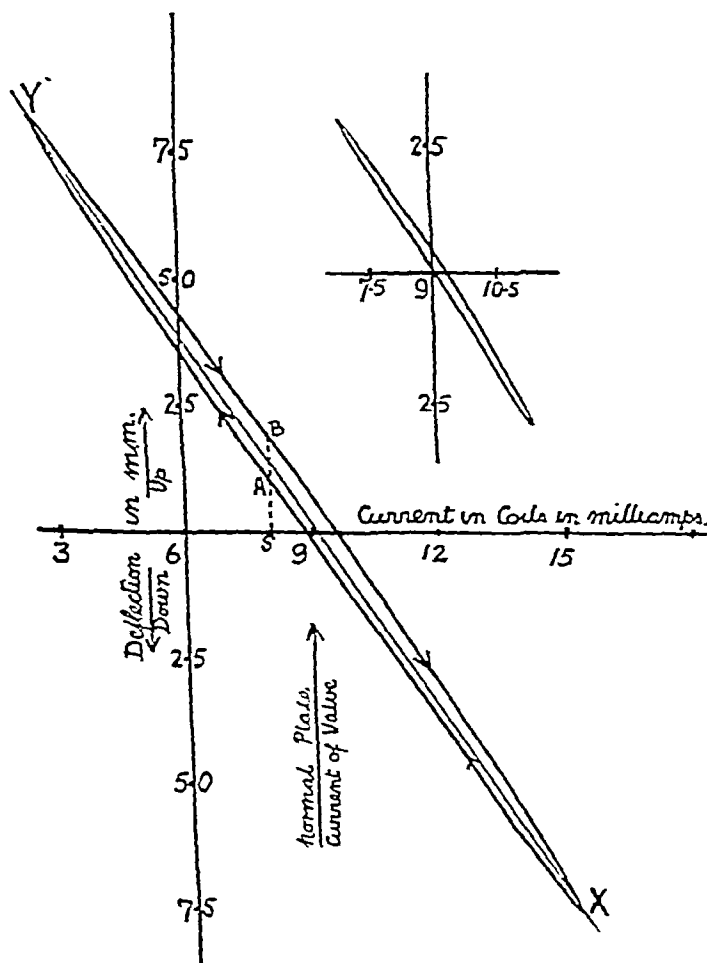


Fig. 6. Hysteresis loops of the pole pieces of the oscillograph.

When the current has a value  $S$ , the deflection will be that corresponding to either  $AS$  or  $BS$  according to whether current is altering from  $X$  to  $Y$  or  $Y$  to  $X$ , that is it depends on the previous deflection.

If however for simplicity it is assumed that the deflection occurs along a straight line  $XY$  it will be seen that the error introduced by this assumption will never be more than 3 p.c. of any deflection.

This last is the only deviation from direct proportion that occurs under all four headings, and therefore not more than 3 p.c. error is introduced by the assumption that the deflection of the tongue is a linear function of the applied potential.

#### OPTICAL AND RECORDING SYSTEM.

Fig. 7 is a diagram of the optical system and arrangements for photography. The heat from the arc is removed by passing the beam through

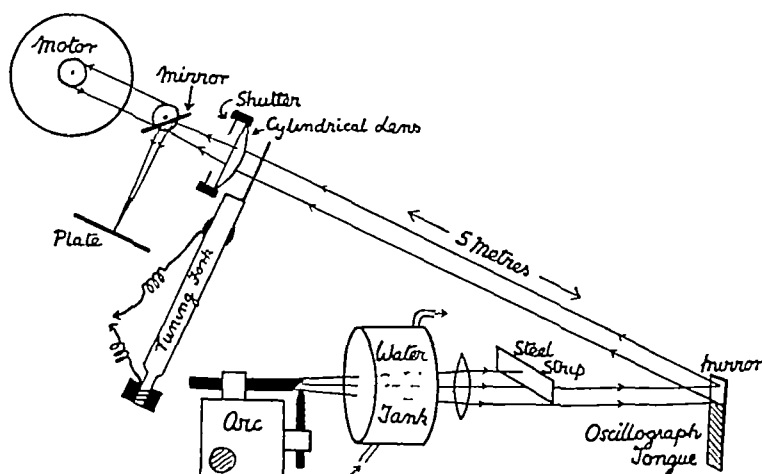


Fig. 7. Diagram of the optical and recording systems.

a glass tank of flowing water before it falls on the mirror mounted on the oscillograph tongue. This mirror is concave and has a focal length of 1 metre. A horizontal steel strip is fixed in a position between the tank and the mirror such that an image of one edge of it is formed at a distance of about 5 metres from the mirror.

After passing through a cylindrical lens the light falls on a second mirror, and is thence reflected on to a photographic plate. The cylindrical lens focusses the primary image into a vertical line on the plate.

The shadow is displaced in a vertical plane by any movement of the oscillograph tongue, and is moved across the plate by the rotation of the second mirror; the latter is mounted on a vertical shaft, driven by an electric motor. In this way the shadow will trace out a curve on the

plate of which ordinates represent potentials, and abscissæ represent time. The time marker is an electrically maintained tuning fork.

When the plate is 5 metres from the oscillograph a movement of 0.005 mm. of the centre of the tongue displaces the shadow 10 mm. This extremely simple optical system produces a good image even at this distance. It is convenient to be able to view the excursion before a plate is exposed, and this can be done by putting a ground-glass screen in the place of the plate. The ground glass is viewed from behind, and owing to the persistence of vision, any deflection appears as a standing curve. When the deflection can be repeated a number of times a contact breaker on the mirror shaft is used to synchronise the excursion with the rotation of the mirror. When records are required over some length of time the plate and rotating mirror are replaced by a strip of cinema film, driven by a clockwork motor at any speed from 10–50 cm. per sec.

#### THE AMPLIFIER.

*Construction.* The amplifier designed to work with this oscillograph follows standard practice in the use of resistance capacity coupling. For convenience it has been made up as two units. The potentials to be measured are applied to the input of the first unit, which consists of 3 valves in cascade. The output is connected to the input of the second unit, in which one amplifying valve is followed by two power valves in parallel; these valves have an internal resistance of 6000  $\omega$  and can carry large plate currents; this is necessary as the oscillograph is a current operated instrument and the present model requires about

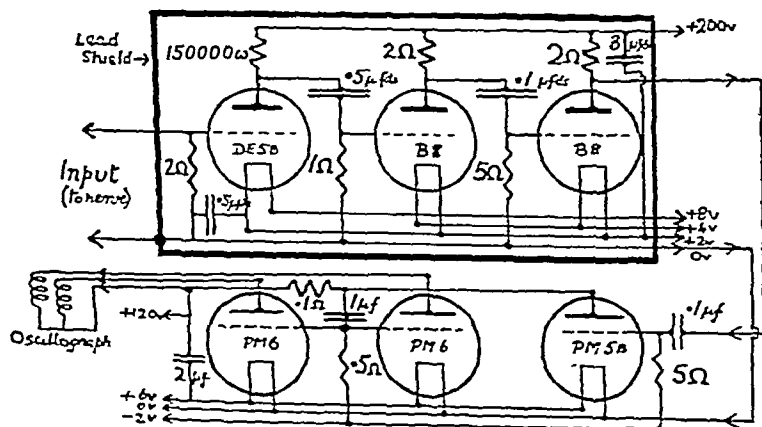


Fig. 8. Diagram showing wiring of the amplifiers and values of the components used.

8 milliamperes for a 10 mm. deflection; the instrument coils are connected directly in the plate circuits of the power valves.

Fig. 8 shows the wiring of the amplifier, and the values of various resistances and condensers; the components used are those sold for use in wireless sets.

The first amplifying unit is contained without its batteries in a wood case  $3'' \times 10'' \times 5''$ ; the battery output and input leads are brought to sockets in a small ebonite panel in one side of the box and contact is made with these by means of small metal plugs.

*Shielding.* The valves are mounted in spring holders, and the whole case filled with small pieces of sponge rubber. The outside of the case is covered with  $1/8$  inch sheet lead connected to earth.

The batteries are not shielded but stand with the amplifier on a wooden tray resting on an inflated motor tyre.

The electrical shielding is all that can be desired, for a small induction coil can be run 12 inches from the amplifier case without producing any disturbance in the record. Complete mechanical shielding has not yet been secured and the instrument has at present to be worked in a quiet room<sup>1</sup>.

It has been found unnecessary to shield the second amplifier at all, and its components are fixed on a wooden tray, protected from dust by a metal lid. Separate batteries are used for the two amplifiers; a battery of small accumulators supplies the high tension current of the second unit, as the large plate currents would rapidly exhaust dry cells. In the first unit a Marconi DE5B valve is followed by two B.T.H. B8 valves; the latter have a very high amplification factor, and the overall amplification is about  $\times 14,000$ . The full amplification is often not required, and can be greatly reduced by cutting out the third valve; the amplification can be varied over a considerable range by altering the voltage of the high tension battery.

*Distortions.* Amplifiers are subject to various distortions (see Adrian (4) and Gasser and Erlanger (5)), and the magnitude of these must be determined for any amplifier before it can be used with confidence.

There are two chief distortions which must be considered, those of the valves themselves and those of the intervalve couplings. The former can be almost entirely eliminated by applying a grid bias to the valves

<sup>1</sup> Since going to press a Cosmos AC/G valve has been used in the first stage of the amplifier. This valve is practically non-microphonic, and with it the mechanical shielding is amply sufficient. The use of this valve also increases the amplification.

such that under working conditions no grid current flows and the valve is working on the straight line portion of its characteristic curve. The characteristic and grid current curves were plotted for each of the valves used in the present amplifier, and it was found that with a negative grid bias of 2 volts throughout these distortions were negligible.

There are two distortions due to the intervalve couplings. First, the charge on the grids is drained away through the grid leaks, so that with the couplings used in the present amplifier the deflection falls to 90 p.c. after 0.05 sec.; but the error is negligible when recording nerve action potentials, the duration of which is of the order 0.002 sec.

When any potential change is impressed on the input of the amplifier, the capacity of the coupling condensers causes an appreciable time to elapse before the potentials of these condensers reach their new values. With the present amplifier this time is less than 0.0001 sec.

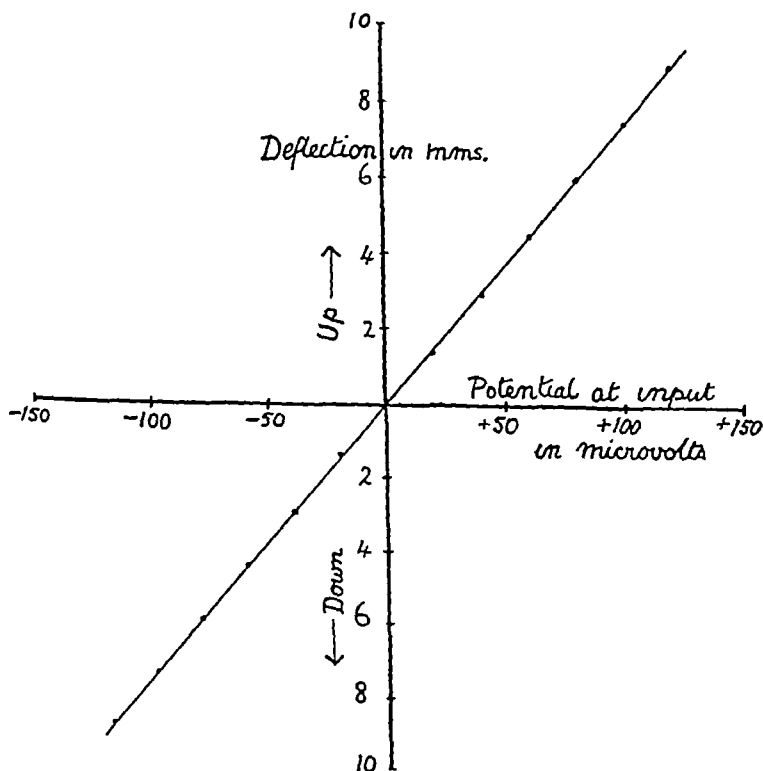


Fig. 9. Graph showing the relation of the height of the deflection reached after 0.001 sec. to the potential producing it.

## CALIBRATION.

The whole distortions of the complete system, amplifier and instrument together, can be determined by comparing known potential changes at the input with their records on photographic plates. The following figures show the results of such a calibration.

The first (Fig. 9) shows that the height of the excursion is directly proportional to the potential at the input. Fig. 10 shows that when the

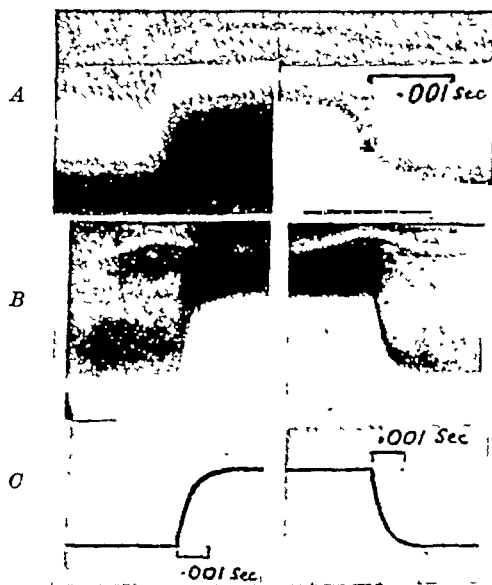


Fig. 10.

- A. Record of instantaneous potential changes applied to the amplifier input; the tongue is damped in oil. The deflection reaches 95 p.c. of its full value in under 0.0002 sec.
- B. Records of potential changes when a condenser ( $1.96 \mu\text{fd.}$  capacity) is charged and discharged through a  $200 \omega$  resistance.
- C. Calculated potential changes of the above discharge on the same scale.

The curves B and C can be exactly superimposed.

The potential rises to 95 p.c. of final value in 0.001 sec., i.e. at about the same rate as the rising phase of action potential of a nerve at  $18^\circ\text{C.}$

Time marker gives 200 D.V. per sec.

tongue is damped in oil the deflection reaches 95 p.c. of its final value in less than 0.0002 sec. and there is no overshwing.

A potential which changes at about the same rate as the action

potential of a nerve can be artificially produced by charging or discharging a suitable condenser through a resistance, and the shape of this potential change can be exactly calculated. Such a potential change can be recorded by the oscillograph, and the theoretical curve compared with the curve thus obtained. Fig. 10, *B* and *C*, shows such a comparison; the curves can be superimposed. The distortion of an action potential from a nerve at  $0^{\circ}$ – $20^{\circ}$  C. will therefore be negligible, and at higher temperatures very small.

#### ELECTRICAL SAFEGUARDS.

When used with the amplifier the instrument cannot be broken by the accidental application of a large potential to the input. If this occurs the tongue is prevented from excessive bending by coming into contact with the pole pieces, and the coils cannot be burnt out as the plate current of the last valve can only rise to the saturation value, which is insufficient to damage the windings. The instrument is thus electrically unbreakable and is also mechanically very robust.

#### SPECIMEN RECORDS OF ACTION POTENTIALS IN NERVE.

The following figures are given as specimen oscillograph records of action potentials. All are taken from the frog's sciatic at room temperature.

Fig. 11 shows action potentials evoked by electrical stimulation. The leads from the nerve were pencils of cotton wool planted in U tubes full of Ringer's fluid into which dipped silver wires coated with silver chloride. The nerve was stimulated through wire electrodes connected to a coreless induction coil, the primary circuit of which was broken by the contact breaker on the rotating mirror shaft. The amplification was reduced by plugging out valve 3 of the first unit, and by replacing the 100,000  $\omega$  anode resistance of valve 1 of the second unit with one of 30,000  $\omega$ .

*A* and *B* are typical diphasic and monophasic records.

*C* and *D* are records of monophasic action potentials from the same nerve taken with the proximal electrode at different distances from the stimulating electrodes. The spreading of the wave which Gasser and Erlanger<sup>(1)</sup> have shown to be due to differences in the rate of conduction in different fibres can be seen from these records. Unfortunately bull frogs were not available, and the effect is much less marked in the short length of nerve obtainable from *Rana temporaria*.



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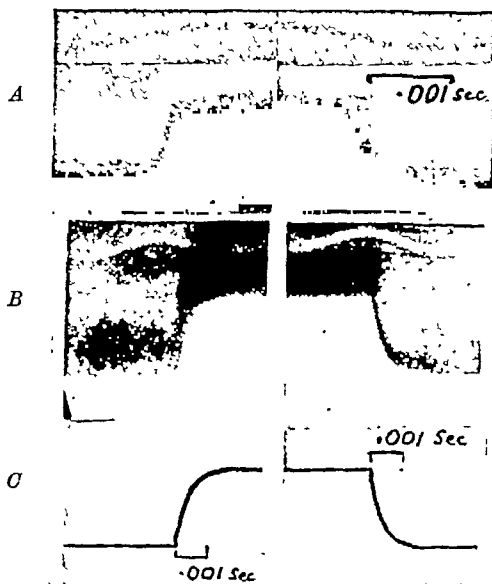


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The potential rises to 95 p.c. of final value in 0.001 sec., i.e. at about the same rate as the rising phase of action potential of a nerve at 18°C.

Time marker gives 200 D.V. per sec.

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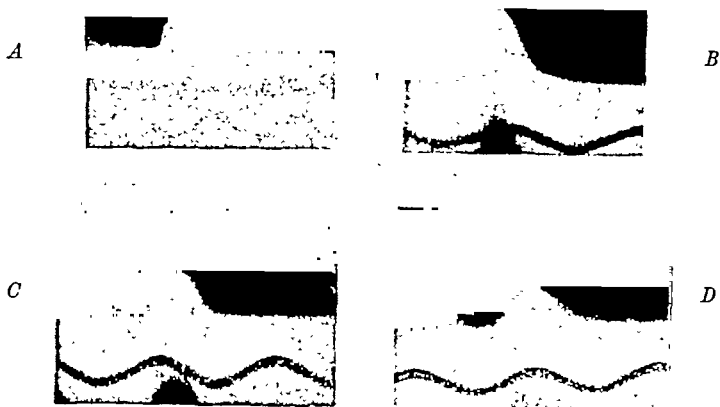


Fig. 11. Records of action potential in frog's sciatic nerve on electrical stimulation. Temp. 12° C.

A. Diphasic, leads 12 mm. apart, proximal electrode 5 mm. from stimulating electrodes.

B. Monophasic, proximal electrode 14 mm. from stimulating electrodes.

C. Monophasic response at 4 mm. from stimulating electrodes.

D. Monophasic response at 29 mm. from stimulating electrodes. Showing greater duration of response as distance from the stimulating electrodes is increased.

Time marker gives 200 D.V. per sec.

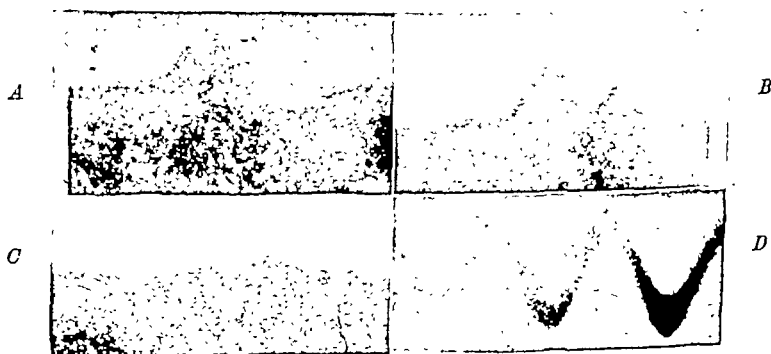


Fig. 12. Records of action potential in frog's sciatic nerve on stretching gastrocnemius by a weight. (Enlarged three times.) Temp. 11° C.

A. Nerve uninjured (diphasic) electrodes 26 mm. apart, single impulse.

B. Nerve injured (monophasic); two impulses at an interval of about 0.0035 sec.

C. Control, nerve killed between muscle and electrodes.

D. Time marker gives 200 D.V. per sec.

Fig. 12 shows action potentials produced by stimulation of sensory endings in the gastrocnemius.

A silk thread was attached to the tendon of the muscle and a small weight was lowered on to this by means of a lever controlled by a treacle dash-pot; the plates were exposed after an interval of 30 seconds, when the initial outburst of impulses had nearly subsided. These records are essentially similar to those which Adrian showed to be due to the activity of single nerve fibres and their time relations agree with his analysed capillary electrometer records (4).

It is interesting to note the brief duration of the crest of the monophasic wave, as Gasser and Erlanger (5) predicted that this would be the case for the potential wave in a single fibre (axon potential wave).

In Fig. 13 are shown records obtained by leading off from a small

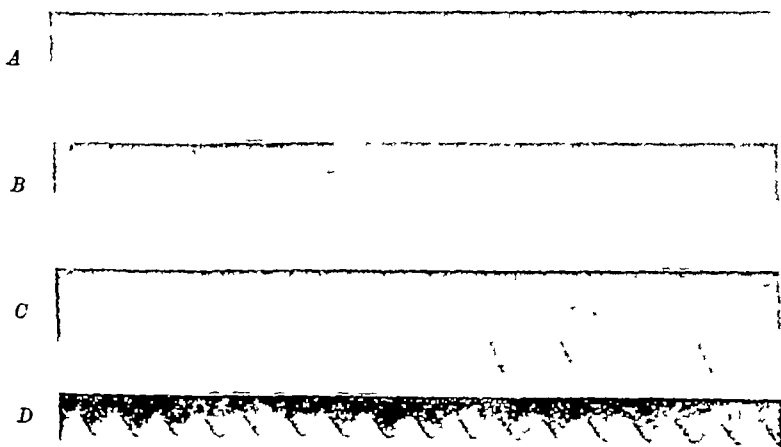


Fig 13. Records of action potentials produced by stimulation of sensory endings in a toe muscle of the frog.

A. Nerve uninjured (diphasic). Electrodes 13.5 mm. apart. 10.2° C. Load 5 grams.

B. Nerve injured (monophasic). 10.2° C. Load 5 grams

C. Nerve uninjured (diphasic). Electrodes 16 mm. apart (reversed to A and B). 12.5° C. Load 5 grams.

D. Tuning fork 200 D.V. per sec.

Records A and B show the rhythmic discharge of a single proprioceptor end organ. In B one impulse from some second end organ also appears.

nerve supplying one of the toe muscles of the frog when that muscle is stretched. A and B show clearly the rhythmic discharge of a single

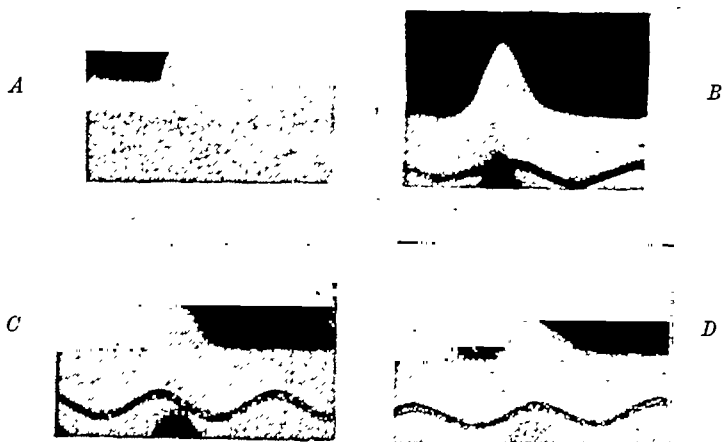


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A. Diphasic, leads 12 mm. apart, proximal electrode 5 mm. from stimulating electrodes.

B. Monophasic, proximal electrode 14 mm. from stimulating electrodes.

C. Monophasic response at 4 mm. from stimulating electrodes.

D. Monophasic response at 20 mm. from stimulating electrodes. Showing greater duration of response as distance from the stimulating electrodes is increased.

Time marker gives 200 D.V. per sec.



Fig. 12. Records of action potential in frog's sciatic nerve on stretching gastrocnemius by a weight. (Enlarged three times.) Temp. 11° C.

A. Nerve uninjured (diphasic) electrodes 26 mm. apart, single impulse.

B. Nerve injured (monophasic); two impulses at an interval of about 0.0035 sec.

C. Control, nerve killed between muscle and electrodes.

D. Time marker gives 200 D.V. per sec.

# THE INFLUENCE OF THE PROTEIN INTAKE ON THE BASAL METABOLISM.

By GEORGE MACFEAT WISHART.

*(From the Institute of Physiology, University of Glasgow.)*

IN view of Rubner's theory of secondary dynamic action of protein, and the frequent observation that the human basal metabolic rate is increased by liberal protein diet, the parallelism (noted in a previous paper<sup>(1)</sup>) between the daily variations in urinary nitrogen and variations in basal metabolism in one subject are, perhaps, not very surprising. As this subject, Wn., had partaken of somewhat abnormal diets, and the correlation between urinary nitrogen and basal metabolism was a close one (correlation coefficient + 0.70), it was thought worth while to confirm the relationship in another subject, whose diet should be kept as normal as possible, consistent with wide variations in the protein component.

The subject was the author, and two series of observations were made. During the first series, lasting 20 days, the diet was uncontrolled, the object being to discover whether the slight daily fluctuations in nitrogen intake on ordinary diet would show any relation to the daily variations in basal metabolism. A second series was then carried out over a period of 40 days; during this period the protein quota of the diet was varied from approximately 30 gm. per diem to 150 gm. per diem. In addition to the estimation of basal metabolism and the total urinary nitrogen, daily observations of the urea, ammonia, creatinine, uric acid, and phosphate-ratio of the urine were made. These subsidiary determinations were done in the hope that, if, on the whole, the basal metabolism was found to be correlated with the total nitrogenous metabolism, deviations from this general rule would be found to be associated with a change in the excretion of some particular nitrogenous urinary constituent.

*Methods.* The following methods were used:

Basal metabolism ...	Douglas Bag and Haldane Air Analysis.
Total nitrogen ...	Kjeldahl.
Urea ...	Urease.

end-organ in response to a steady stimulus. Adrian has shown this to occur with several types of sensory end organ. These records were taken on bromide paper bent in an arc which replaced the plate; in this way a well-focussed image can be obtained over a considerable length of record, which is impossible on a flat plate.

#### SUMMARY.

The paper describes a new form of oscillograph and an amplifier adapted to work with it. This oscillograph is capable of giving more accurate records of nerve action potentials than have been obtained with any other instrument except the cathode ray oscillograph, and it has the advantage over this that it can give a record of a single impulse in one nerve fibre. The instrument is electrically unbreakable. The possible sources of error are discussed, and it is shown by calibration curves that the distortions are very small. The photographic records of this oscillograph suffer from errors of less than 3 p.c. and are in linear coordinates. Some specimen records are given of action potentials from nerves, both when stimulated electrically and through their receptor organs.

I wish to express my warmest thanks to Dr E. D. Adrian for his constant encouragement and advice throughout this work, the expenses of which have been defrayed by a grant to him from the Government Grants Committee of the Royal Society.

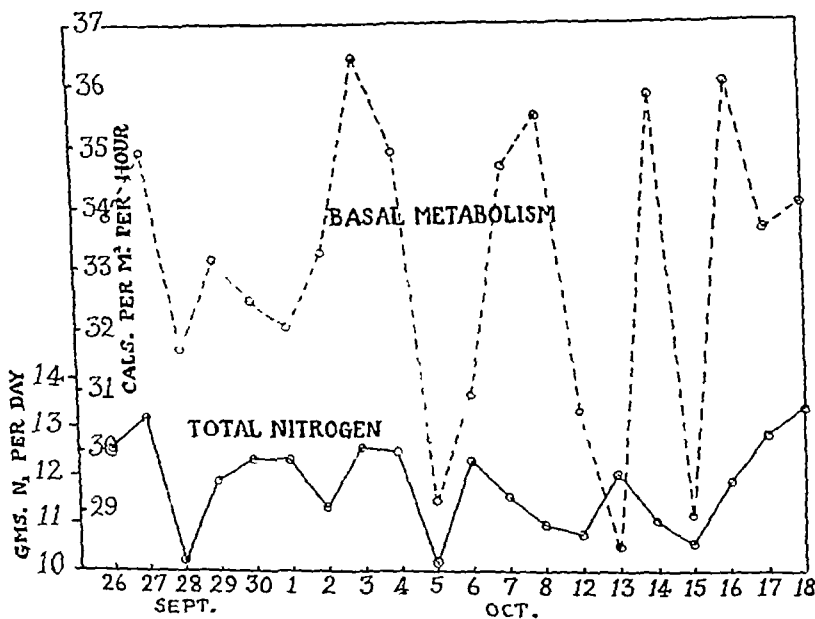
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RESULTS.

*First Series.*

The 20 consecutive daily observations on normal diet of the first series showed that (1) the fluctuations of urinary nitrogen on uncontrolled diet were comparatively small (max. 13.44 gm., min. 10.08 gm.) and (2) afforded indications of some parallelism between nitrogenous output and basal metabolic rate. Graphical evidence of this parallelism is given in Graph I.



Graph I.

The degree of similarity between the two curves may be given a numerical value by calculating the statistical correlation coefficient  $r$ . This was found to be  $r_{B.T.N.} = +0.41 \pm 0.125$ , a significant correlation. The correlation coefficient between basal metabolic rate and the urinary excretion of urea was almost as high— $r_{B.Urea} = +0.38 \pm 0.129$ . Correlation between each of the other nitrogenous constituents determined and the basal metabolism gave coefficients which were insignificant in comparison with their probable errors.



Ammonia ...	...	Folin.
Uric acid ...	...	Hopkins-Folin.
Creatinine ...	...	Folin.
Phosphate-ratio ...	...	Leathes.

The sample of expired air for the basal metabolism estimation was collected by a skilled operator immediately before the subject rose in the morning at 8 a.m. The urine was collected from 8 a.m. to 8 a.m., and, throughout the following discussion, the basal metabolic data are compared with the urinary data for the collection ending at the time of the basal estimation. A short series of unpublished observations, wherein the estimation of the basal metabolism took place at the mid-point of the urine collection period, seemed less likely to yield fruitful results, and was abandoned.

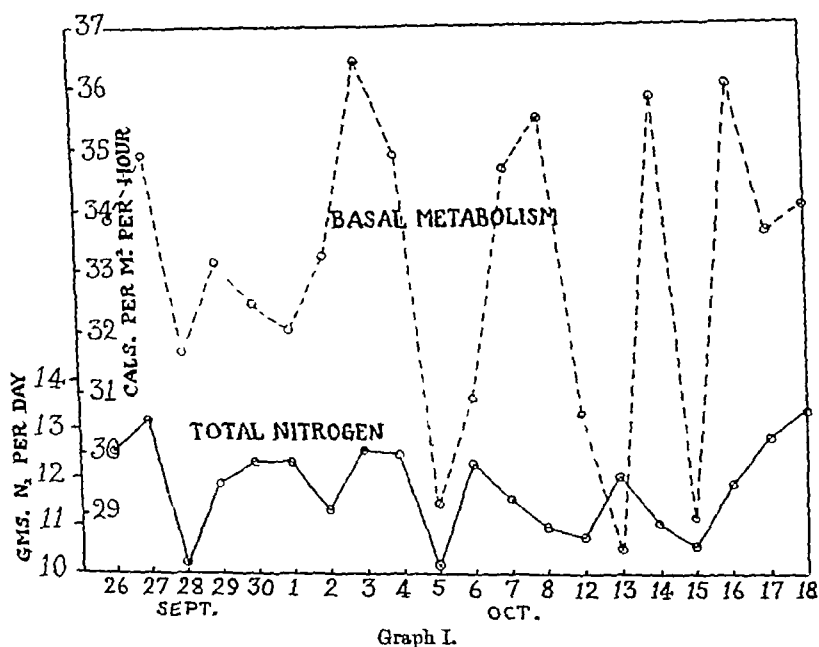
Before commencing the experiment, it was realised that, in an investigation of this kind, the greatest possible accuracy of technique was essential. The most extreme care was therefore taken with all the analyses, each recorded datum being the average of two agreeing analyses; wherever the slightest doubt existed as to the technical accuracy of an analysis, the analysis was repeated. On December 1st, in the second series, owing to a leaky valve, the collection of expired air was faulty and all data for this day had to be omitted. This omission along with a day's interval between the normal diet period and the first period of low protein diet were the only breaks in an otherwise complete set of consecutive daily observations.

The dietary during the first series was the subject's ordinary everyday diet. During the second series the dietary arrangements were as follows: (1) 6 days on normal diet as in first series; (2) 11 days on a fixed diet containing about 30 gm. protein per diem; (3) 12 days on a fixed diet containing about 150 gm. protein per diem; and (4) 11 days' repetition of (2). The low-protein diet was composed of bread, potatoes, butter, tapioca, and syrup, and had a calorie value of approximately 2900. In the high-protein diet the tapioca and syrup were omitted and eggs, ham and meat substituted, the calorie value being approximately 3100. The extra 200 calories were found to be necessary to prevent loss of body-weight. On the whole, the body-weight remained fairly constant throughout the experiment, varying from 64.0 kilos on the first day to 61.6 kilos on the last day of the second series.

# RESULTS.

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*Second Series.*

Since, in the first series, variations due to errors of estimation were large in comparison with the nitrogen fluctuations, more striking results were expected from the second series, in which the protein intake was altered as widely as possible without making the diet excessively abnormal.

The fluctuations in urinary nitrogen and basal metabolism are shown in Graph II. The transient fall in basal metabolism reported by Borgstrom, Hafkesbring and Bost(2), as following increased nitrogenous metabolism has not been confirmed, the curves showing a parallel relation to one another, similar to, but more marked than in the case of the first series.

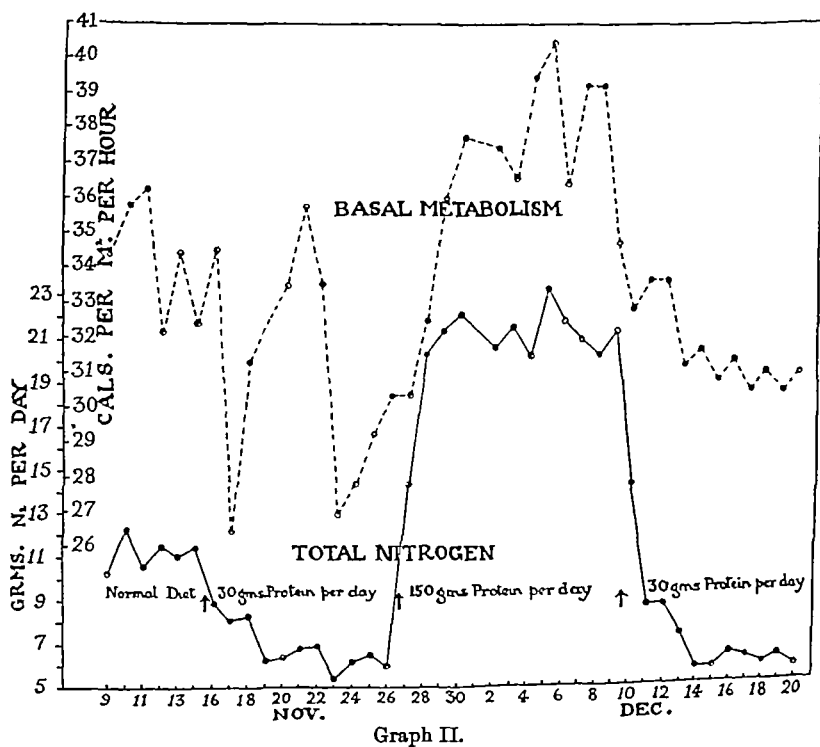


Table I gives the coefficients of correlation between the basal metabolism and (1) the total nitrogen, and (2) each of the other urinary constituents estimated.

TABLE I. (40 observations.)

Correlated variables	Correlation coefficient
B.M. and total nitrogen	$r_{N.T.N} = +0.75 \pm 0.047$
B.M. and urea	$r_{N.Urea} = +0.75 \pm 0.047$
B.M. and ammonia	$r_{N.Amm.} = +0.71 \pm 0.053$
B.M. and creatinine	$r_{N.Creat.} = +0.76 \pm 0.045$
B.M. and uric acid	$r_{N.Uric\ Ac.} = +0.73 \pm 0.050$
B.M. and acidity*	$r_{N.Acidity} = +0.44 \pm 0.086$

\* As expressed by phosphate-ratio.

Since, as may be observed from the graph, there is a distinct tendency for the changes in basal metabolism to lag behind the changes in urinary nitrogen, correlation coefficients were also calculated for the series of 31 observations remaining after the arbitrary exclusion of three consecutive days at each of the three transition periods when dietary changes were made. These values are given in Table II.

TABLE II. (31 observations.)

Correlated variables	Correlation coefficient
B.M. and total nitrogen	$r_{N.T.N} = +0.84 \pm 0.036$
B.M. and urea	$r_{N.Urea} = +0.84 \pm 0.036$
B.M. and ammonia	$r_{N.Amm.} = +0.76 \pm 0.051$
B.M. and creatinine	$r_{N.Creat.} = +0.82 \pm 0.040$
B.M. and uric acid	$r_{N.Uric\ Ac.} = +0.85 \pm 0.034$
B.M. and acidity*	$r_{N.Acidity} = +0.59 \pm 0.079$

\* As expressed by phosphate-ratio.

## DISCUSSION OF RESULTS.

### A. Correlation between Basal Metabolism and Nitrogen Excretion.

There exists a remarkable parallelism between the urinary nitrogen excretion and the basal metabolic rate, as evidenced by the graphs and the high correlation coefficients of  $+0.84$  for the 31 days and  $+0.75$  for all 40 days. A much higher degree of correlation would have resulted in the absence of the curiously aberrant values of 18th to 22nd November; no emotional or other cause could be determined to account for these high values. This relation between urinary nitrogen and basal metabolism is evident even in the slight fluctuations occurring on a normal uncontrolled diet.

Comparing the coefficients for all 40 observations with those obtained after exclusion of the transitional periods, the latter are seen to be not only actually higher in every case, but also greater in proportion to their probable errors.

Moreover, in each series, with the exception of the acidity coefficient, the correlation between basal metabolism and each nitrogenous constituent is of approximately the same value; this excluded any possibility of demonstrating, as had originally been hoped, that those basal metabolism values which were aberrant in relation to the total nitrogen excretion would be found to be associated with some marked deviation in the excretion of one particular nitrogenous component. To determine whether such aberrant values were associated with alterations in urinary acidity, as estimated by the phosphate-ratio, the partial correlation coefficient between basal metabolism and acidity, nitrogen being kept constant, was calculated. The coefficient so obtained is an indication of any association of changes in basal metabolism with urinary acidity, when the average variations of each with the nitrogen excretion are eliminated. This partial correlation coefficient was  $r_{B, A_{\text{cd}}} = -0.28 \pm 0.112$ ; it was small in comparison with its probable error, and is therefore of doubtful significance.

#### B. *Prediction in the Individual of the Basal Metabolism from the Urinary Nitrogen Excretion.*

As a test of the value of the statistical correlation between total nitrogen and basal metabolism, one may attempt to predict the latter from the former. Assuming straight-line regression, the regression equation calculated from the series of 31 observations (transitional periods excluded) was

$$x = 28.456 + 0.449y,$$

where  $x$  = basal metabolism in cal. per sq. meter per hour,

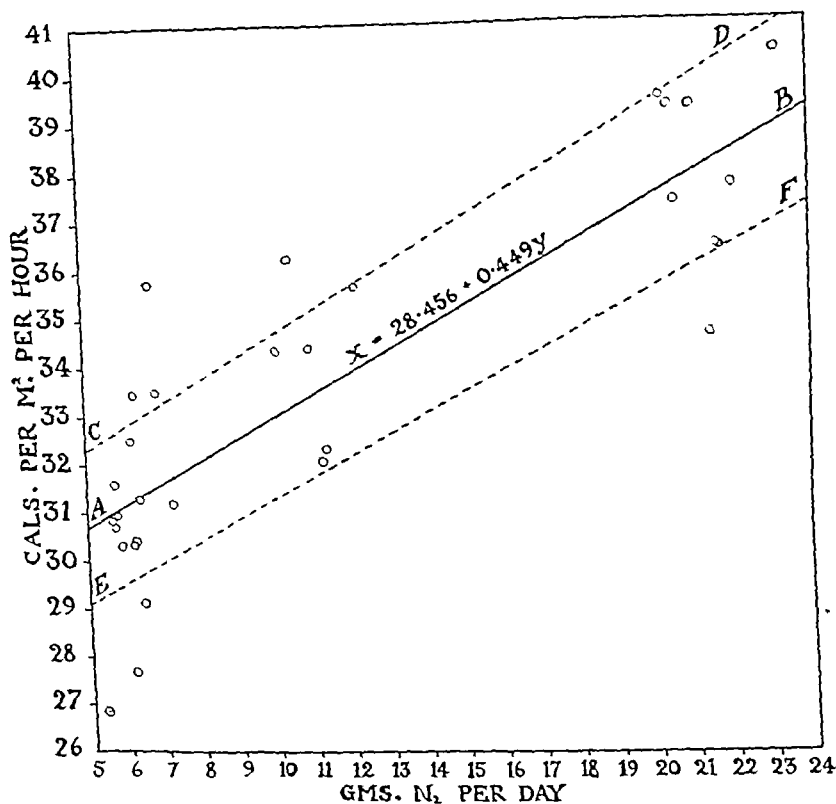
and  $y$  = urinary total nitrogen in gm. per day.

If the basal metabolism be expressed, not as per unit area, but in total calories per hour, the equation becomes

$$x = 52.645 + 0.831y.$$

In this subject, therefore, the basal metabolic rate altered by about eight-tenths of a calorie per hour or by about half a calorie per hour when the metabolism was related to his surface-area, for a change of 1 gm. in the urinary nitrogen excretion. The graphic representation of these equations gives, for this subject, the average value of the basal metabolism associated with any particular value of urinary nitrogen. Graph III is a "scatter" diagram showing the relation of the actually observed values to these average values as represented by the regression line  $AB$ . Lines  $CD$  and  $EF$  are drawn at distances from  $AB$

equivalent to a deviation of the basal metabolism by 5 p.c. Of the eight observations lying outwith this range, six occurred on consecutive days.



Graph III.

Such results indubitably show that, in assessing the value of the normal basal metabolism of an individual, the level of the protein intake must be taken into account. It is doubtful if this has been sufficiently appreciated, except by Krogh, who demands that the subject shall subsist on a low protein dietary for two days previous to the basal estimation (3).

In the writer's experiments the difference between the metabolism as observed, on the day following the lowest nitrogen excretion (5.292 gm.), and the day after the highest nitrogen output (23.240 gm.), was 13.56 calories per square metre per hour or 51 p.c. of the lower value. Using the predicted values from the regression equation for such

nitrogen excretions, the difference would be 26 p.c. The protein intake is therefore a factor of very considerable importance in modifying the basal metabolic rate.

### C. Correlation in Other Subjects.

Similar correlations have been calculated for three other subjects in whom, in this laboratory, both urinary nitrogen and basal metabolism had been determined daily over fairly long periods. Unfortunately, in only one of these (Wn.—male), who was frequently altering his diet for other experimental purposes, were there any great fluctuations in nitrogen output. The other two (D.—male, and Be.—female), on the contrary, were on fixed diet and endeavouring to maintain their nitrogen metabolism at constant level. The results were as follows:

TABLE III.

Subject	Wn.	D.	Be.
No. of observations	77	41	31
Correlation coefficient $r_{N.T.N.}$	$+0.70 \pm 0.05$	$+0.30 \pm 0.096$	$+0.15 \pm 0.118$

It will be observed that, in Wn., in whom the nitrogenous intake fluctuated widely, a coefficient, approximating to that obtained in the writer as subject, was found, and, even in the other two subjects, D. and Be., in whom, on account of the constant diet, little correlation would be expected, the coefficients were both positive in trend though only one, D., was significant in relation to its probable error. This tendency to parallelism between nitrogenous and basal metabolism is apparently, therefore, a general one, and may be rendered evident, by statistical treatment of a series of observations, even in the comparatively minute fluctuations of nitrogen output that occur on a constant diet.

### D. Prediction of the Basal Metabolism from the Urinary Nitrogen in Different Individuals.

The existence of a positive correlation in all the subjects examined suggested that one of the factors, and possibly the main factor, in the variations in basal metabolic rates of different individuals lay in differences of nitrogenous metabolism. This idea gained much support when it was found that the regression equation calculated from the observations on Wn. was  $x = 28.269 + 0.448y$ , being thus almost identical with the equation  $x = 28.456 + 0.449y$  obtained from the observations on the writer as subject. This similarity in the reactions of Wn. and the writer to alterations in nitrogenous metabolism is shown in another

way in Table IV, wherein the data from Wn. are divided up into dietary periods, during each of which the nitrogen output remained fairly constant. For each of these periods, the basal metabolism as observed on Wn. is compared with the metabolism as computed from the nitrogen output by the regression equation developed from the metabolism values of the writer.

TABLE IV.

Urinary nitrogen gm. per day	B.M. observed on Wn.	B.M. calculated from writer's data	Percentage difference
3.80	30.6	30.2	-1.3
3.86	29.7	30.2	+1.7
6.46	30.5	31.4	+2.9
7.25	31.7	31.7	0.0
9.24	32.7	32.6	-0.3
12.46	34.6	34.1	-1.5
13.89	34.2	34.7	+1.4
16.52	36.6	35.9	-1.9

The similarity between these two subjects was so close that it seemed highly improbable that it was due to mere coincidence. It should be noted, however, that both subjects are assistants in this laboratory; their daily work and mode of life were similar, and their calorie intakes almost identical when expressed per unit of surface-area.

The validity of the prediction formula was then tested on the other subjects examined in this laboratory for whom suitable data were available. In applying such regression equations to the computation of the basal metabolism of different individuals, one may use either the formula  $28.456 \div 0.449y$ , which deals with the metabolism as expressed per unit of surface, or the formula  $52.645 \div 0.831y$ , which gives results in terms of total metabolism and takes no account of the surface-area at all. Moreover, it was hoped that, by finding which formula had the better prediction value, some indication would be obtained of whether the basal metabolism was a function of the surface-area, or was entirely a matter of the "active protoplasmic mass," as is Benedict's view. In the latter case, since the nitrogen excretion alone is presumably an indication of the amount of "active protoplasmic mass," one would expect better prediction results from the formula  $52.645 \div 0.831y$ , in which considerations of surface-area are omitted.

In the following table (V) the actual average metabolic rates of four subjects of this laboratory for whom the necessary data were available, are compared with the predicted values obtained by each of the two formulæ. One female subject has been included, though the justification for this is obviously somewhat doubtful.



TABLE V.

Subject	A	B	Percentage difference A and B	C	D	Percentage difference C and D
	Observed metabolism per sq. m. per hour	Calculated metabolism per sq. m. per hour		Observed total metabolism per hour	Calculated total metabolism per hour	
Wn. (male)*	31.7	31.7	0.0	53.6	58.7	- 8.7
D. (male)	37.9	35.3	+ 7.4	64.8	65.3	- 0.8
M. (male)	36.7	32.8	+ 11.9	59.4	60.7	- 2.1
Be. (female)	31.2	33.3	- 6.4	51.8	61.8	- 16.2
	Average variation + 3.2			Average variation - 6.0		

\* Period of normal diet only.

It is rare to find in the literature instances of simultaneous observation of urinary nitrogen and basal metabolism where the daily data are given, but the prediction values of both formulæ were also tested on (1) results reported by Borgstrom, Hafkesbring and Bost(4), and (2) data taken from the classical paper by Benedict, Miles, Roth and Smith(5) on under-nutrition. The application of the formulæ to such data as could be obtained from these papers showed divergences between predicted and observed values even greater than those given in the table above. In view of the excellent results obtained by the application of the formulæ to subject Wn., the results on other subjects in this laboratory and on the American subjects were therefore surprisingly disappointing in their irregularity.

Further, while it has been a general experience in this laboratory to obtain results considerably below the Benedict or Du Bois' standards, a difference probably mainly associated with the fact that all our subjects were trained laboratory workers and thoroughly accustomed to metabolic measurements, even when allowance is made for this difference, the predicted values afford no indication as to whether the basal metabolism is primarily determined by the "active protoplasmic mass" or by surface-area.

The unsatisfactory results obtained by the application of the prediction formulæ to such data as were available suggests that differences in nitrogenous metabolism will not alone account for the differences in basal metabolic rates of different individuals. In the two subjects, Wn. and the writer, in whom good agreement was obtained, the calorie intakes per unit of surface-area were almost identical. In the work of Benedict and his co-workers on under-nutrition previously referred to, despite a fall in urinary nitrogen per man per day of only 1.5 gm., restriction of the calorie value of the diet by approximately 30 to 40 p.c. produced an average fall in the basal metabolic rate of about 20 p.c. Habitual differences in the calorie intake of different individuals might

therefore quite well act as an additional factor in occasioning differences in their resting metabolisms.

*E. Difference in Behaviour of the Nitrogenous Urinary Constituents at the Dietary Transition Periods.*

A minor point of interest which might prove worthy of further investigation is the different behaviour of the various nitrogenous constituents of the urine when abrupt changes in the protein intake were made. The two extremes of this different behaviour are exhibited. on the one hand, by uric acid, which moves immediately, in the course of the first day after the dietary change is made, either up or down to its new value; and, on the other hand, by the ammonia which shows a gradual progressive change to its new level. The urea and creatinine excretion show changes of a type intermediate between these two extremes.

SUMMARY.

1. A marked parallelism exists between the daily variations in basal metabolism and the variations in the output of nitrogen in the urine. This relation has been demonstrated in several subjects, even in the slight fluctuations that occur on normal diet, but has been much more strikingly shown by a series of daily observations on the writer, during which his protein intake was extensively altered.

2. The correlation between urinary nitrogen and basal metabolism in this series was remarkably close; when expressed statistically, the coefficient of correlation was  $\div 0.84 \pm 0.036$ .

3. This statistical relationship was used for the prediction of the basal metabolism in the writer, at any given level of nitrogenous metabolism, with fairly satisfactory results.

4. Basal metabolism values predicted from the same formula showed remarkably good agreement with the observed value in another subject whose protein intake had been extensively varied.

5. General application of the formula to the prediction of the basal metabolism of a number of individuals, both of this and other laboratories, from their average protein metabolism, met with little success.

6. In the writer, the fluctuations of basal metabolism with alterations in protein intake were considerable. The lowest value observed, when the subject was on a diet containing 30 gm. protein per day, was 26.83 cal. per square metre per hour; the maximum value, when the diet contained 150 gm. protein per day, was 40.39 cal. per square metre

per hour. This emphasises the need for something approaching steady dietary conditions previous to basal metabolism estimations.

It is a pleasure to record my indebtedness to Prof. E. P. Clegg for his helpful advice and criticism during the course of this work.

#### REFERENCES.

1. Wishart. *Quart. Journ. Med.* 20. p. 203. 1927.
2. Borgstrom, Hafkesbring and Bost. *Amer. Journ. Physiol.* 79. p. 245.
3. Krogh. *C.-R. Soc. Biol.* 87. p. 458. 1922.
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5. Benedict, Miles, Roth and Smith. *Carnegie Inst. Washington, Pub. No. 238.*

# TISSUE OXYGEN TENSION AND HÆMOGLOBIN.

By J. ARGYLL CAMPBELL.

(*From the National Institute for Medical Research, Hampstead.*)

IN recent researches(1, 2) the relationships between tissue  $O_2$ -tensions and  $O_2$ -pressures in the inspired air were studied. The main object of the present experiments is to determine the influence of variations of hæmoglobin percentage, such as occur in anæmias and other blood diseases, upon tissue  $O_2$ -tensions.

*Technique.* The tissue  $O_2$ -tensions were estimated by the method (3) of injection of nitrogen—about 200 to 300 c.c.—under the skin and into the abdominal cavity of rabbits and drawing off samples after constancy of tensions had become established in the injected gas, no anæsthetics being required. The animals were kept in their usual cages with a plentiful supply of water and fed on a liberal diet of hay, grain (oats), and fresh cabbage.

The hæmoglobin percentage was altered by five different methods, three of them producing a marked decrease, and two a marked increase. The former methods were (1) injection of hæmolytic serum, (2) bleeding from an ear vein and (3) previous prolonged exposure of the rabbits to abnormally high  $O_2$ -pressure in the inspired air; whilst the latter methods were (1) injection of whole blood obtained from other rabbits, and (2) previous prolonged exposure of the rabbits to low  $O_2$ -pressure in the inspired air.

For hæmolysis of red cells, the hæmolytic serum was prepared in a goat by my colleagues Major Dunkin, Dr P. P. Laidlaw and Dr P. Hartley; I am indebted to them for an efficient product. Washed red blood corpuscles from a rabbit were made into a 20 p.c. emulsion in normal saline and injected subcutaneously into a goat, the injections being continued until the serum of the goat showed a hæmolytic titre of about 1/100 c.c. The goat was then bled to the extent of about 250 c.c. and the clear serum pipetted off, placed in sterile phials each of about 2.5 c.c. capacity and kept at about 4° C. Two sera were tried, one slightly weaker than the other, the stronger being employed in most of the experiments.

For experiment the hæmolytic serum was warmed and then injected

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For experiment the hæmolytic serum was warmed and then injected

into an ear vein of a rabbit. The outline of eight experiments is given in Table I.

TABLE I. Experiments with hæmolytic serum.

Rabbit Ref. No.	Weight grm.	Hæmolytic serum injected intravenously (ear)
18	3000	1 c.c. on 1st day and 0.75 c.c. on 2nd day; weaker serum; died
17	2950	1 c.c. on 1st day and 0.5 c.c. on 3rd day; weaker serum; died
20	3000	1 c.c. on 1st day and 0.25 c.c. on 3rd day; weaker serum; died
25	1860	0.5 c.c. on 1st day; stronger serum; recovered
26	2090	0.4 c.c. on 1st day and 0.6 c.c. on 2nd day; stronger serum; recovered
22	2260	0.4 c.c., 0.2 c.c. and 0.25 c.c. on 1st, 4th and 6th days respectively; stronger serum; recovered
23	2270	0.25 c.c., 0.12 c.c. and 0.2 c.c. on 1st, 4th and 6th days respectively; stronger serum; recovered
33 (S)	2510	0.25 c.c., 0.15 c.c., 0.5 c.c. and 1.0 c.c. on the 1st, 3rd, 4th and 5th days respectively; stronger serum; died

(S) = splenectomised.

Four animals survived and full details were obtained from them, the red cells being hæmolyzed gradually so that the hæmoglobin per-

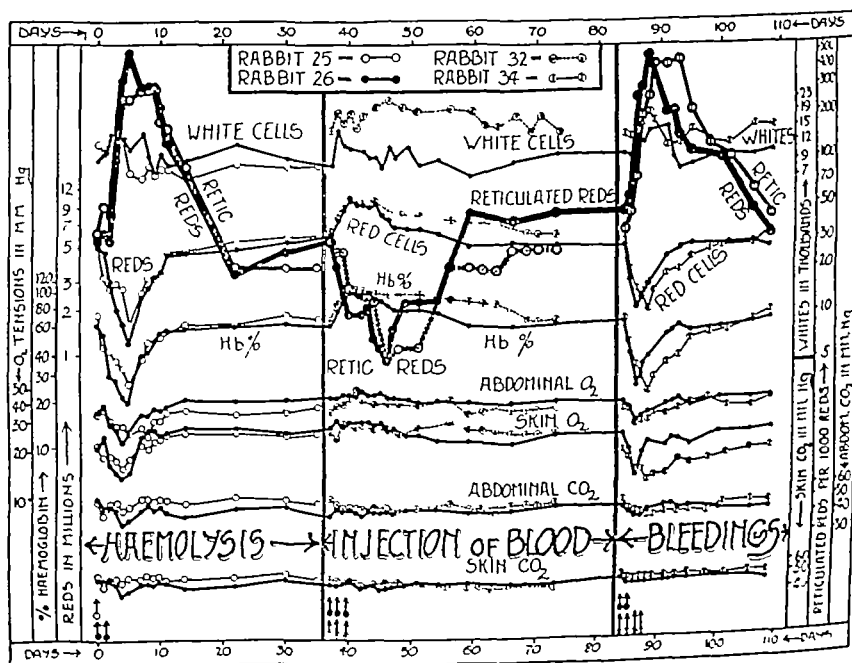


Fig. 1. Details of example of experiments. Hæmolytic serum: Rabbit 26, two injections of hæmolytic serum; Rabbit 25, one injection. Injection of blood: Rabbits 26 and 32, three injections each. Bleedings: Rabbit 26, two bleedings; Rabbit 34, four bleedings. The arrows at the base line indicate the days when the injections and bleedings were carried out.

centage and the red cell counts reached their minima in about 5 to 7 days whilst regeneration of red cells required from about 8 to 20 days before the normal red cell count was reached again. Fig. 1 gives details from two of the rabbits, namely, Nos. 25 and 26. The time factors for red cells are similar to some of those given by Muir and McNee(4). The surviving animals appeared little disturbed by the experiment, the rectal temperature remaining almost normal, and there was only a loss of about 200 grm. in body weight, a loss which was soon made good whilst the red cells were regenerating.

In five further experiments the hæmoglobin was lowered by bleeding, four of the animals surviving (Table II). Usually the animals were bled from an ear vein by about one-third of their total volume on each of three consecutive days, the total blood volume being taken as about 5 p.c. of the body weight(5). The animal, rabbit No. 25, which was bled to a greater extent than this died after the second bleeding.

TABLE II. Bleeding experiments.

Rabbit Ref. No.	Weight grm.	Amount of blood obtained from ear vein
38	3300	45 c.c., 55 c.c. and 35 c.c. on 1st, 2nd and 3rd days respectively
34 (S)	2500	34 c.c., 40 c.c., 30 c.c. and 30 c.c. on 1st, 2nd, 3rd and 4th days respectively
37	3060	60 c.c., 50 c.c., 40 c.c., 35 c.c. and 30 c.c. on 1st, 2nd, 3rd, 4th and 5th days respectively
26	2850	50 c.c. on 1st day and 60 c.c. on 2nd day
25	2610	60 c.c. on 1st day and 60 c.c. on 2nd day; died 1 hour after 2nd bleeding

(S) = splenectomised.

In this series the hæmoglobin percentage and the red cell counts reached their minima after the last bleeding, that is in from 2 to 5 days; regeneration required about 9 to 26 days before the red cell counts returned to normal level, thus resembling in time characteristics the changes produced by hæmolysis. The details of the experiments with rabbits Nos. 26 and 34 are shown in Fig. 1, the results obtained for changes in hæmoglobin percentage resembling some of those of Boycott and Douglas(6).

Three experiments were performed in which the hæmoglobin percentage and red cells of a rabbit were markedly increased by intravenous injection into an ear vein of blood from other rabbits (Table III). To prevent clotting 0.5 grm. sodium citrate was placed in a sterile receptacle for each 70 c.c. of blood, the animals being bled aseptically from an ear vein. The injections were carried out slowly under slight positive pressure, the blood being kept warm (37° C.) throughout by previous



TABLE III. Experiments with injection of large quantities of whole blood.

Rabbit Ref. No.	Weight gm.	Amount of blood injected intravenously (ear)
26	2595	70 c.c. (Hb p.c. = 62), 40 c.c. (Hb p.c. = 50) and 65 c.c. (Hb p.c. = 58) on 1st, 2nd and 3rd days respectively
25	2420	50 c.c. (Hb p.c. = 60), 75 c.c. (Hb p.c. = 60) and 30 c.c. (Hb p.c. = 57) on 1st, 2nd and 3rd days respectively
32 (S)	2530	50 c.c. (Hb p.c. = 60), 50 c.c. (Hb p.c. = 57) and 45 c.c. (Hb p.c. = 64) on 1st, 2nd and 3rd days respectively
		(S) = splenectomised.

enclosure in an incubator and, during injection, by means of a warm-water jacket. The details of the experiments with rabbits Nos. 26 and 32 are given in Fig. 1. Boycott and Douglas(7) proved that after injection of whole blood, the excess plasma is rapidly absorbed whilst the red cells remain in circulation and thus greatly increase the concentration of hæmoglobin percentage and red cells per c.mm., the blood volume remaining practically at normal level. The animal then gradually removes the excess corpuscles, 14 to 28 days being expended in my experiments on this process. Rabbit No. 26 was employed in each of the three types of experiment just described (Fig. 1) but sufficient time was allowed to elapse between the experiments to enable the animal to return to almost normal condition.

In some of my previous experiments(1, 2) in which animals were exposed for prolonged periods to decrease of  $O_2$ -pressure in the inspired air, it was found that the increases in hæmoglobin percentage and in red cells produced by the  $O_2$ -deficiency, persisted for a few weeks after the return to normal  $O_2$ -pressure in the air again; these increases in hæmoglobin percentage produced, whilst breathing  $O_2$  at normal pressure, definite increases in the tissue  $O_2$ -tension of seven rabbits, which had not been very seriously affected by the previous exposure to the low  $O_2$ -pressure in the air. This method of increasing hæmoglobin percentage has the advantage that the increase is produced by the same rabbit itself and not by injection of "foreign" blood from another rabbit. However, there was no striking difference between the effects upon  $O_2$ -tensions in the tissues, produced by these two methods, and also the times required to remove the excess corpuscles were similar in both.

In other previous experiments(1, 2) two rabbits had been exposed for prolonged periods to abnormally high  $O_2$ -pressure in the air which caused a marked decrease in hæmoglobin percentage; when these animals were exposed to normal  $O_2$ -pressure in the air again, their tissue  $O_2$ -tensions were found to be subnormal.

*Effects upon blood.*

The main effects of these experimental procedures are grouped together in Table IV, the maxima or minima for the various factors being alone considered therein. In Table V details are given of the times required from the first day of each experiment to produce the effects shown in Table IV. Complete details of blood changes in two examples of each experiment are given in Fig. 1.

In the hæmolytic experiments the red cells were reduced on an average from 4.9 millions per c.mm. to 1.6 (Table IV), a decrease of about 67 p.c., whilst the average decrease in hæmoglobin percentage was from 60 to 26, a decrease of about 57 p.c. In the bleeding experiments the red cells were decreased on an average from 5.5 millions per c.mm. to 1.8 (Table IV), and the hæmoglobin percentage from 68 to 21, representing decreases of about 67 p.c. and 70 p.c. respectively. In accordance with the findings of previous workers attempts to reduce the hæmoglobin percentage further were unsuccessful, so that the limits obtained probably represent the lowest compatible with life.

TABLE IV. Blood changes; main effects of hæmolysis, bleeding and injection of blood.

Rabbit Ref. No.	Experiment	Red cells, millions per c.mm.		Hb p.c.		Colour index		Ret. red cells per thousand red cells		White cells, thousands per c.mm.	
		Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.
13 (died)	Hæmolysis	5.0	1.0	7.1	15	1.00	1.01	20	—	15.2	—
25	"	5.2	1.6	72	26	1.00	1.18	33	300	11.5	12.6
17 (died)	"	4.9	2.5	70	42	1.00	1.18	32	—	10.5	—
26	"	5.0	1.2	63	20	1.00	1.32	30	500	9.0	13.5
22	"	5.1	2.0	58	31	1.00	1.36	28	520	10.8	20.5
20 (died)	"	4.2	2.2	55	32	1.00	1.11	75	—	10.6	—
23	"	4.2	1.7	46	27	1.00	1.45	27	400	13.5	21.0
	Av. for survivors	4.9	1.6	60	26	1.00	1.33	29	430	11.2	16.9
38	Bleeding	6.2	1.6	74	20	1.00	1.05	22	450	10.3	12.9
34 (S)	"	5.8	1.9	67	23	1.00	1.05	35	430	13.5	18.0
37	"	5.2	1.6	67	17	1.00	0.82	52	530	12.2	17.9
26	"	5.0	2.0	63	25	1.00	1.00	45	470	9.6	14.0
25 (died)	"	6.0	3.0	76	35	1.00	0.92	20	100	10.2	15.6
	Av. for survivors	5.5	1.8	69	21	1.00	0.98	38	470	11.4	15.7
26	Injection of blood	6.0	10.5	63	102	1.00	0.77	30	5	9.1	14.0
25	"	6.0	11.0	69	112	1.00	0.83	20	3	8.4	12.5
32 (S)	"	5.3	10.0	63	106	1.00	0.82	30	5	14.0	18.0
	Av. for survivors	5.8	10.5	67	107	1.00	0.82	27	4	10.5	14.8

(S) = splenectomised.

In the experiments of the opposite nature, where large quantities of blood were injected intravenously the average increase in red cells per c.mm. was from 5.8 to 10.5 millions (Table IV), the average increase

TABLE III. Experiments with injection of large quantities of whole blood.

Rabbit Ref. No.	Weight gm.	Amount of blood injected intravenously (ear)
26	2595	70 c.c. (Hb p.c.=62), 40 c.c. (Hb p.c.=50) and 65 c.c. (Hb p.c.=58) on 1st, 2nd and 3rd days respectively
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enclosure in an incubator and, during injection, by means of a warm-water jacket. The details of the experiments with rabbits Nos. 26 and 32 are given in Fig. 1. Boycott and Douglas(7) proved that after injection of whole blood, the excess plasma is rapidly absorbed whilst the red cells remain in circulation and thus greatly increase the concentration of hæmoglobin percentage and red cells per c.mm., the blood volume remaining practically at normal level. The animal then gradually removes the excess corpuscles, 14 to 28 days being expended in my experiments on this process. Rabbit No. 26 was employed in each of the three types of experiment just described (Fig. 1) but sufficient time was allowed to elapse between the experiments to enable the animal to return to almost normal condition.

In some of my previous experiments(1, 2) in which animals were exposed for prolonged periods to decrease of  $O_2$ -pressure in the inspired air, it was found that the increases in hæmoglobin percentage and in red cells produced by the  $O_2$ -deficiency, persisted for a few weeks after the return to normal  $O_2$ -pressure in the air again; these increases in hæmoglobin percentage produced, whilst breathing  $O_2$  at normal pressure, definite increases in the tissue  $O_2$ -tension of seven rabbits, which had not been very seriously affected by the previous exposure to the low  $O_2$ -pressure in the air. This method of increasing hæmoglobin percentage has the advantage that the increase is produced by the same rabbit itself and not by injection of "foreign" blood from another rabbit. However, there was no striking difference between the effects upon  $O_2$ -tensions in the tissues, produced by these two methods, and also the times required to remove the excess corpuscles were similar in both.

In other previous experiments(1, 2) two rabbits had been exposed for prolonged periods to abnormally high  $O_2$ -pressure in the air which caused a marked decrease in hæmoglobin percentage; when these animals were exposed to normal  $O_2$ -pressure in the air again, their tissue  $O_2$ -tensions were found to be subnormal.

*Effects upon blood.*

The main effects of these experimental procedures are grouped together in Table IV, the maxima or minima for the various factors being alone considered therein. In Table V details are given of the times required from the first day of each experiment to produce the effects shown in Table IV. Complete details of blood changes in two examples of each experiment are given in Fig. 1.

In the hæmolytic experiments the red cells were reduced on an average from 4.9 millions per c.mm. to 1.6 (Table IV), a decrease of about 67 p.c., whilst the average decrease in hæmoglobin percentage was from 60 to 26, a decrease of about 57 p.c. In the bleeding experiments the red cells were decreased on an average from 5.5 millions per c.mm. to 1.8 (Table IV), and the hæmoglobin percentage from 68 to 21, representing decreases of about 67 p.c. and 70 p.c. respectively. In accordance with the findings of previous workers attempts to reduce the hæmoglobin percentage further were unsuccessful, so that the limits obtained probably represent the lowest compatible with life.

TABLE IV. Blood changes; main effects of hæmolysis, bleeding and injection of blood.

Rabbit Ref. No	Experiment	Red cells, millions per c.mm.		Hb p.c.		Colour index		Ret. red cells per thousand red cells		White cells, thousands per c.mm.	
		Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.
18 (died)	Hæmolysis	5.0	1.0	74	15	1.00	1.01	20	—	15.2	—
25	"	5.2	1.6	72	26	1.00	1.18	33	300	11.5	12.6
17 (died)	"	4.9	2.5	70	42	1.00	1.18	32	—	10.5	—
26	"	5.0	1.2	63	20	1.00	1.32	30	500	9.0	13.5
22	"	5.1	2.0	58	31	1.00	1.36	28	520	10.8	20.5
20 (died)	"	4.2	2.2	55	32	1.00	1.11	75	—	10.6	—
23	"	4.2	1.7	46	27	1.00	1.45	27	400	13.5	21.0
Av. for survivors		4.9	1.6	60	26	1.00	1.33	29	430	11.2	16.9
38	Bleeding	6.2	1.6	74	20	1.00	1.05	22	450	10.3	12.9
34 (S)	"	5.8	1.9	67	23	1.00	1.05	35	430	13.5	18.0
37	"	5.2	1.6	67	17	1.00	0.82	52	530	12.2	17.9
28	"	5.0	2.0	63	25	1.00	1.00	45	470	9.6	14.0
25 (died)	"	6.0	3.0	76	35	1.00	0.92	20	100	10.2	15.6
Av. for survivors		5.5	1.8	68	21	1.00	0.98	38	470	11.4	16.7
28	Injection of blood	6.0	10.5	63	102	1.00	0.77	30	5	9.1	14.0
25	"	6.0	11.0	69	112	1.00	0.68	20	3	8.4	12.5
32 (S)	"	5.3	10.0	68	106	1.00	0.52	30	5	14.0	16.0
Av. for survivors		5.8	10.5	67	107	1.00	0.82	27	4	10.5	14.5

(S) = splenectomised.

In the experiments of the opposite nature, where large quantities of blood were injected intravenously the average increase in red cells per c.mm. was from 5.8 to 10.5 millions (Table IV), the average increase

in hæmoglobin percentage being from 67 to 107; these figures indicate increases of 81 p.c. and 60 p.c. respectively. Similar increases were observed in the experiments in which hæmoglobin percentage and red cells were increased by exposure to low  $O_2$ -pressure in the air(1, 2). Taking all the above results together the changes in hæmoglobin percentage obtained varied from about 70 p.c. below normal to about 60 p.c. above normal, and the main object of the present research was to determine the effects of such marked changes in hæmoglobin percentage upon tissue  $O_2$ -tensions; but before proceeding to the study of this question, there are several other points of interest concerning the blood which may be noted briefly here.

As regards the colour index, hæmolysis produced a marked increase on an average from 1.00 to 1.33 (Table IV) resembling some figures given by Muir and McNee(4), the explanation probably being the appearance of red cells of large size in the circulation and perhaps also some of the hæmoglobin from the hæmolysed cells had been taken up by the surviving red cells. Bleeding produced no marked changes in the colour index (Table IV) either immediate or delayed. In injection of blood there was an apparent immediate decrease in colour index (Table IV), but this was merely due to the fact that in the injected blood the red cells contained less hæmoglobin than did those of the host.

In hæmolysis and bleeding the main after-effects concern the regeneration of blood during which new red cells are rapidly freed into the circulation as indicated by the marked increase in number of red cells, particularly of reticulated red cells; these latter are now generally regarded as young red cells. In hæmolysis the reticulated red cells increased from a normal average of 29 per 1000 red cells to a maximum average of 430 per 1000 red cells (Table IV), whilst in the bleeding experiments the increase was from the normal average of 38 per 1000 to the maximum average of 470 per 1000 red cells. Similar increases following recovery from marked anæmias have been observed by Key(6) and others. My maximum figures are high probably because of the conditions of the experiments, and my animals were young growing animals. In Table V the times to produce the maximum effects and the duration in days from the beginning of the experiment to the day when the blood regained normal characteristics are stated, so that by subtracting one from the other, the period in days required for regeneration of blood may be obtained; this period varied considerably in different animals, in hæmolysis the average regeneration periods for red cells and hæmoglobin percentage being 12.5 and 13.7 days respectively,

TABLE V. Blood changes; number of days since 1st day of experiment to produce (1) maximum effect and (2) return to normal.

Rabbit Ref. No.	Experiment	Red cell counts			Hb p.c.			Ret. red cells			White cells		
		Max.	Re- turn to normal	Diff.	Max.	Re- turn to normal	Diff.	Max.	Re- turn to normal	Diff.	Max.	Re- turn to normal	Diff.
25	Haemolysis	5	22	17	5	30	25	8	23	14	5	6	1
26		5	22	17	5	22	17	5	23	17	3	8	5
27		6	14	8	6	11	5	7	19	12	6	8	2
23		6	14	8	6	14	8	7	22	25	6	8	2
		Av. 5.5	18.0	12.5	5.5	19.2	13.7	6.7	23.7	17.0	5.0	7.5	2.5
35	Bleeding	3	29	26	3	23	20	9	23	14	3	5	2
34 (S)		4	24	20	4	21	17	9	24	15	4	7	3
37		5	18	13	4	16	14	7	20	13	4	11	6
26		2	11	9	2	9	7	4	21	17	7	8	1
		Av. 3.5	20.5	17.0	3.2	17.7	14.5	7.2	22.0	14.7	4.5	7.7	3.0
26	Injection of blood	3	17	14	6	22	16	9	29	20	1	6	5
25		3	29	26	7	29	22	10	36	26	5	6	1
32 (S)		5	31	26	4	31	27	9	29	20	9	24	15
		Av. 4.3	25.7	22.0	5.6	27	22	9.3	31	22	5	12	7

(S) = splenectomised.

whilst in the bleeding experiments the average regeneration periods were 14.5 days for hæmoglobin percentage and 17.0 days for red cells; the average periods for return of reticulated red cell counts to normal were not very different, namely 17.0 days in the hæmolytic experiments and 14.7 days in the bleeding experiments.

The average total number of red cells destroyed by hæmolysis was 3.2 millions per c.mm. (Table VI). There is no evidence that during

TABLE VI. Hæmolysis—regeneration of red cells per diem and per c.mm.

Rabbit Ref. No.	Total No. of red cells destroyed by hæmolytic serum, millions per c.mm.	Av. increase in red cells per diem; thousands per c.mm.	Av. No. of ret. red cells counted per diem in excess of normal; thousands per c.mm.
25	3.6	210	270
26	3.8	220	280
27	3.1	390	460
23	2.5	310	470
	Av. 3.2	282	370

hæmolysis the total blood volume is greatly changed so that consideration of the total number of red cells destroyed per c.mm. gives an accurate indication of the total number of red cells destroyed in the whole blood. To replace this 3.2 millions of red cells per c.mm., the red cell counts were increased on an average by 282,000 per c.mm. per diem, and the average of reticulated red cells counted per diem in excess

of normal was 370,000 per c.mm. during the same period of regeneration (Table VI).

In the bleeding experiments the average total number of red cells removed was 3.8 millions per c.mm. (Table VII). Boycott and

TABLE VII. Bleedings—regeneration of red cells per diem and per c.mm.

Rabbit Ref. No.	Total No. of red cells removed by bleeding; millions per c.mm.	Av. increase in red cells per diem; thousands per c.mm.	Av. No. of ret. red cells counted per diem in excess of normal; thousands per c.mm.
38	4.6	180	360
34 (S)	3.9	190	490
37	3.6	230	560
26	3.0	330	620
Av.	3.8	245	507

(S)=splenectomised.

Douglas(9) did observe an increase in total blood volume, but only in some animals, following bleeding, but the error will not be great for our purpose if we neglect the total blood volume and consider only the number of red cells per c.mm. To replace the 3.8 millions per c.mm. removed by bleeding, an average increase of 245,000 per c.mm. per diem of red cells was noted (Table VII) and the average number of reticulated red cells counted in excess of normal per diem was 507,000 per c.mm. during the period of regeneration.

Using the oxygen capacity as an indirect means of estimation Boycott and Douglas(10) state that the average life of a red cell is about 25 days. The normal number of reticulated red cells counted per diem in a rabbit's normal blood may be taken as about 35 per 1000 red cells. If we take the normal red cell count as 5.0 millions per c.mm. all these red cells could be replaced in about 28 days if a reticulated red cell is a young red cell and exists as such for one day only; this 28 days agrees closely with the above observed 25 days. To return to the abnormal condition, when there was a marked increase (0.5 to 1.0 million per c.mm.) in red cell count on any one day, at the early stage of regeneration following bleeding, the increase in reticulated red cells for the same day accounted for the whole increase in red cells; but later the calculations became complicated, and it was evident that many of the reticulated red cells must have remained as such in the circulation for more than one day; they are included more than once in the averages (Tables VI and VII).

With regard to the opposite experiments, when the red cells were greatly increased, the average total number of red cells in excess of

normal was 4.7 millions per c.mm. (Table VIII). In the removal of this excess of red cells a considerable period, 22 days, was expended (Table V) and two processes at least were concerned, namely, decrease in number of new red cells, *i.e.* of reticulated red cells and increase in rate of destruction of red cells. The lowest count observed for reticulated red cells was 3 per 1000 red cells (Fig. 1, Table IV). The reticulated

TABLE VIII. Injection of blood—removal of red cells per diem and per c.mm.

Rabbit Ref. No.	Total No. of red cells in excess of normal due to injection of blood; millions per c.mm.	Av. deficit of ret. red cells per diem; thousands per c.mm.	Av. No. of red cells removed by blood- destroying organs in excess of normal per diem; thousands per c.mm.
26	4.5	80	250
25	5.0	50	140
32 (S)	4.7	50	140
	Av. 4.7	60	170

(S)=splenectomised.

red cells counted per diem were decreased on an average by 60,000 per c.mm., the normal average being about 150,000 per c.mm.; whilst the blood-destroying organs destroyed 170,000 per c.mm. per diem in excess of normal (Table VIII). In making the above calculations no attention has been directed to total blood volume because this remains practically unaltered under the conditions specified.

With regard to white cells (Table IV), there was a temporary increase in numbers in all experiments, *i.e.* after hæmolysis, bleeding and injection of blood; in the latter case the increase was due merely to the white cells added during the injection of blood and to consequent concentration. The increase in leucocytes following hæmolysis was also observed by Muir and McNee(4). The cause for this increase and also for the increase following bleeding is not clear; the differential counts did not reveal any constant or striking increase in any one particular kind of leucocyte. Splenectomised animals Nos. 32, 33 and 34 had a higher leucocyte count under normal conditions than normal animals, but resembled some of the normal animals in all their responses to bleeding and injection of blood.

#### *Effects upon tissue O<sub>2</sub>-tensions.*

The normal figures for hæmoglobin percentage and tissue O<sub>2</sub>-tensions are given in Table IX. All these animals appeared to be in good health and were regarded as normal animals for experimentation, although the



hæmoglobin percentage varied from 95 to 46; the red cell counts showed much smaller variations, namely from 4.2 to 6.2 millions per c.mm. The behaviour of the animals with the lower values of hæmoglobin percentage, during the various experiments did not differ markedly from that of the animals with the much higher values. The most striking thing, however, was that normally, the animals with low hæmoglobin percentage had practically the same tissue  $O_2$ -tensions as the animals with the very high hæmoglobin percentage. Thus, rabbit No. 23 with hæmoglobin percentage 46 gave 25 and 39 mm. Hg for  $O_2$ -tensions under the skin and in the abdominal cavity respectively, practically the same figures as those for rabbit No. 1 with hæmoglobin percentage 95. It seems evident that the volume of blood circulating per unit time was much greater in rabbit No. 23 than in rabbit No. 1. Normal tissue  $O_2$ -tensions—and also tissue  $CO_2$ -tensions—of different rabbits are thus

TABLE IX. Normal figures for Hb p.c. and tissue gas tensions.

Rabbit Ref. No.	Hb p.c.	Tissue $CO_2$ -tensions mm. Hg		Tissue $O_2$ -tensions mm. Hg	
		Skin	Abd. cav.	Skin	Abd. cav.
1	95	50	50	25	38
2	90	47	46	26	44
15	90	53	52	18	38
3	85	38	39	31	42
16	82	50	47	20	42
18	74	45	42	21	41
38	74	49	47	21	35
26	72	53	54	21	35
12	70	49	48	27	37
13	70	50	52	18	38
14	70	47	—	22	—
17	70	50	46	16	35
32 (S)	68	51	56	25	34
33 (S)	68	50	53	23	35
34 (S)	67	53	54	19	35
37	67	53	52	20	38
26	63	51	51	20	35
22	58	53	54	21	35
20	55	55	51	20	38
23	46	49	48	25	39

(S) = splenectomised.

usually much alike, as was shown previously (11), and exhibit a marked degree of independence of normal hæmoglobin percentage. On the other hand, in any one animal marked experimental alterations in hæmoglobin percentage have marked effects upon tissue  $O_2$ -tensions as will be observed in Tables X A and X B, and in Figs. 2 and 3, the main conclusion being that the tissue  $O_2$ -tensions decrease as the hæmoglobin percentage decreases and *vice versa*. In Figs. 2 and 3, the results for each animal

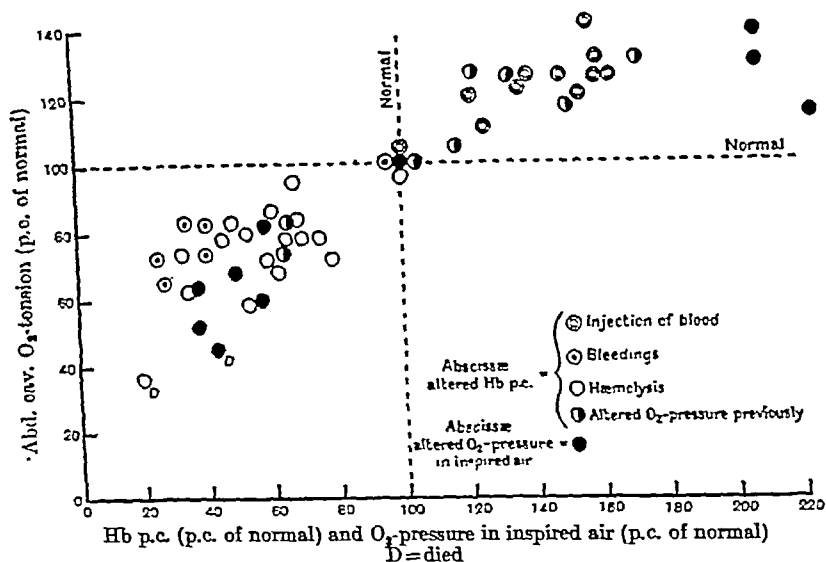


Fig. 2. Composite chart, showing (1) relations between haemoglobin percentage (p.c. of normal) and O<sub>2</sub>-tension in abdominal cavity (p.c. of normal); and (2) relations between O<sub>2</sub>-pressure in the inspired air (p.c. of normal) and O<sub>2</sub>-tension in abdominal cavity (p.c. of normal).

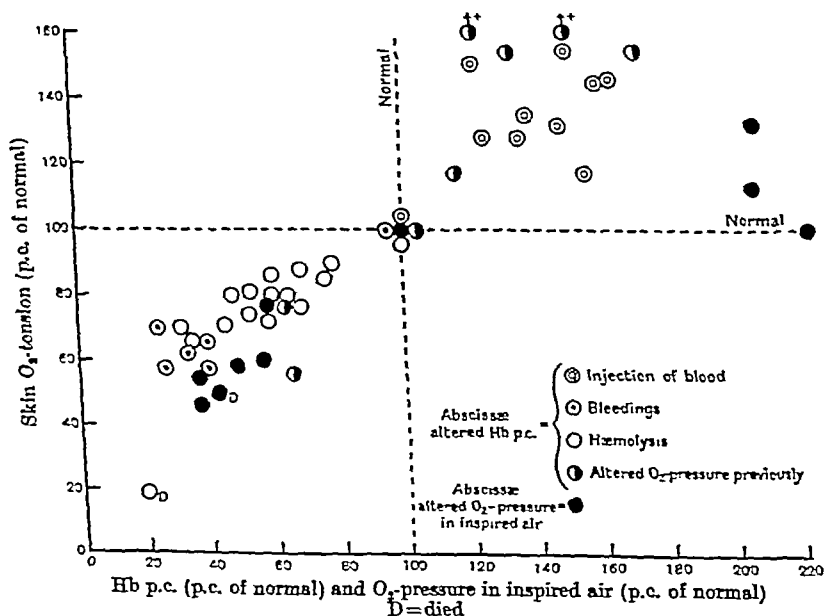


Fig. 3. Composite chart, showing (1) relations between haemoglobin percentage (p.c. of normal) and O<sub>2</sub>-tension under the skin (p.c. of normal); and (2) relations between O<sub>2</sub>-pressure in the inspired air (p.c. of normal) and O<sub>2</sub>-tension under the skin (p.c. of normal).

TABLE X A. Main effects of hæmolysis, bleeding and injection of blood on tissue O<sub>2</sub>-tensions.

Rabbit Ref. No.	Experiment	No. of days to produce effects	Hb p.c.		Tissue O <sub>2</sub> -tensions, mm. Hg			
					Skin		Abd. cav.	
			Before exp.	After exp.	Before exp.	After exp.	Before exp.	After exp.
18 (died)	Hæmolysis	2	74	15	21	4	41	15
17 (died)	"	2	70	42	16	9	35	23
20 (died)	"	2	55	32	15	14	41	34
25	"	5	72	26	21	15	35	24
26	"	5	63	20	20	13	35	22
22	"	6	58	31	20	15	38	22
23	"	6	46	27	25	18	39	28
Av. for survivors			60	26	21	15	37	24
25 (died)	Bleeding	2	76	35	24	14	38	36
38	"	3	74	20	21	12	35	23
34 (S)	"	4	67	23	19	12	35	29
37	"	5	67	17	20	14	38	28
26	"	2	63	25	23	13	39	29
Av. for survivors			68	21	21	13	37	27
26	Injection of blood	6	63	102	25	29	41	46
25	"	8	69	112	25	28	37	45
32 (S)	"	5	68	106	25	29	34	48
Av. for survivors			67	107	25	29	37	46

(S) = splenectomised.

TABLE X B. Tissue O<sub>2</sub>-tension as affected by increase (and decrease) of Hb p.c. due to previous prolonged exposure to low (and high) O<sub>2</sub>-pressure in the inspired air; animal now breathing normal air.

now breathing normal air.				Tissue O <sub>2</sub> -tensions mm. Hg			
Rabbit Ref. No.	No. of days exposure	Hb p.c.		Skin		Abd. cav.	
		Before exposure	Day after termina- tion of exposure	Before exposure	Day after termina- tion of exposure	Before exposure	Day after termina- tion of exposure
Previously exposed to low O <sub>2</sub> -pressure:							
2	43	90	120	26	40	44	55
3	11	85	100	31	37	42	44
12	33	70	120	27	42	37	48
13	33	70	105	18	30	38	44
14	33	70	105	22	34	—	41
15	33	90	110	16	35	33	42
16	33	82	110	20	40	42	46
	Av.	79	110	23	39	39	46
Previously exposed to high O <sub>2</sub> -pressure:							
2	28	90	60	25	14	41	34
3	38	85	55	32	25	43	32
	Av.	87	57	28	19	42	33

are plotted in percentages of the normal figures for purposes of comparison; thus, hæmoglobin percentage 46 and hæmoglobin percentage 95 are placed at the point 100 on the abscissæ when dealing with rabbits Nos. 23 and 1 respectively. Similarly, for the tissue  $O_2$ -tensions, the normal figures for each animal are placed at the point 100 on the ordinates.

Marked decreases of hæmoglobin percentage produced marked decreases of tissue  $O_2$ -tensions; the points for hæmolysis, for bleeding etc. are drawn so as to be distinguishable from one another on the charts. In the more resistant animals the tissue  $O_2$ -tensions fell in percentage about half that of the fall in hæmoglobin percentage; whilst in the less resistant the tissue  $O_2$ -tensions fell relatively about the same as the hæmoglobin percentage; in the more resistant animals probably the heart was able to work more efficiently and to compensate in some degree for the loss of hæmoglobin. Figs. 2 and 3 are of value as indicators of the possible effects upon tissue  $O_2$ -tensions of various degrees of anæmia. It is also obvious from Figs. 2 and 3 and from Tables X A and X B that increase above normal of hæmoglobin percentage caused a definite increase in tissue  $O_2$ -tensions. In Table X A are given the chief details of the experiments of increase of hæmoglobin percentage by injections of blood, whilst the results in Table X B were obtained from experiments in which the hæmoglobin percentage was increased (and decreased) by previous prolonged exposure to low (and high)  $O_2$ -pressure in the air. In the low  $O_2$ -pressure experiments, rabbits Nos. 2 and 3 were exposed to the changes at ordinary barometric pressure in a special closed chamber(1), whilst the other five rabbits were exposed to decompressed air in the decompression chamber at Messrs Siebe Gorman's premises, London; the increases in hæmoglobin percentage with these two methods of exposure to low  $O_2$ -pressure in the air showed no marked difference.

The increase in tissue  $O_2$ -tensions produced by marked increase of hæmoglobin percentage was similar in degree to that produced by a short spell of vigorous exercise or a fit of convulsions(12).

On the same charts (Figs. 2 and 3)—using rabbits with normal hæmoglobin percentage—the results of breathing  $O_2$  at different percentages of the normal pressure, upon tissue  $O_2$ -tensions have also been plotted in order to compare the changes in tissue  $O_2$ -tensions produced by a given percentage fall (or rise) in hæmoglobin percentage with the changes produced by the same percentage fall (or rise) in  $O_2$ -pressure in the air. Figs. 2 and 3 are therefore composite with two sets of abscissæ. If any difference exists at all, the points for the experiments

in which the  $O_2$ -pressure in the air was decreased, lie at a lower level than those for the experiments in which hæmoglobin percentage was decreased. This appears to disprove the existence of secretion of  $O_2$  by the lungs during anoxæmia in any important degree because it could exert its influence in the former case only and tissue  $O_2$ -tensions should therefore be higher. The points while breathing  $O_2$  at low pressure were obtained from rabbits which had several weeks' acclimatisation to low  $O_2$ -pressure in the air; however, acclimatisation in itself has no great influence upon tissue  $O_2$ -tension (1, 2).

In all the experiments it was obvious that the tissue  $O_2$ -tension of rabbits could not be lowered beyond 40 p.c. of normal for any length of time without risk of death. During the most vigorous experimental procedures it is therefore not possible to reduce the tissue  $O_2$ -tension to anywhere near nil, so that it is not reasonable to talk of tissue  $O_2$ -tension as almost non-existent and to attempt to explain growth of anaerobic bacteria in normal tissue, with circulation intact, as due to lack of  $O_2$ . The tissues considered herein, namely, the subcutaneous tissue and the peritoneal endothelium cannot be more exacting than that of most other organs in their demands for  $O_2$ , and it is probable that all other tissues fared at least as well as these two tissues. Recent experiments of Simpson and Macleod (13) and of others prove that the amount of lactic acid is exceedingly small in normal resting muscle if proper technique is used for estimation, so that the  $O_2$ -tension in normal resting muscle is obviously not nil as is often stated.

The lowest  $O_2$ -pressure in the inspired air tested in the previous experiments was about 7 p.c. of an atmosphere (50 mm. Hg) almost equivalent to that at the summit of Mt Everest. From my results with rabbits it would appear that a climber at the summit of Mt Everest is as badly off as regards tissue  $O_2$ -tension as an individual at sea level whose hæmoglobin percentage has fallen to between half and one-third of the normal value.

The great alterations in  $O_2$ -tensions in the tissues produced by alterations in hæmoglobin percentage, undoubtedly constitute one of the chief stimuli by means of which the blood is brought back to normal condition again following hæmolysis, bleeding or injection of blood. There is a normal tissue  $O_2$ -tension and if this is altered the organism, as soon as possible, endeavours to restore the tissue  $O_2$ -tensions to normal level again. For all animals tested, including monkey and man, the normal tissue  $O_2$ -tensions have been found to lie usually between 20 and 40 mm. Hg (11). It is suggested that, in the geological epoch

when tissues were first shut off from the external atmosphere, such tensions (20–40 mm. Hg) of  $O_2$  existed in this atmosphere; a similar hypothesis is advanced for  $CO_2$ -tensions in the tissues, the normal figures for which are usually about 40–50 mm. Hg(1).

Dr H. H. Dale kindly removed the spleens from four rabbits; after about 2 months' rest two were tested for their reactions to bleeding and to injection of blood. As regards tissue  $O_2$ -tensions etc., they behaved just like some of the normal rabbits, any differences being insignificant and explained by differences in weight and in detail of experiment. The results for splenectomised rabbits Nos. 32 and 34 are included in the various figures and tables. More recently hæmolytic has been tested with splenectomised rabbits; rabbit No. 33 (Table I) exhibited an abnormal reaction, the serum failing to produce definite hæmolytic although it was toxic to the animal; but rabbits Nos. 32 and 34 responded just like some of the normal animals, full details however being obtained too late for inclusion in this paper.

### *Effect upon $CO_2$ -tensions.*

The chief details of the effects of alterations of hæmoglobin percentage upon tissue  $CO_2$ -tensions are given in Tables XI A and B and

TABLE XI A. Main effects of hæmolytic, bleeding and injection of blood on tissue  $CO_2$ -tensions.

Rabbit Ref. No.	Experiment	No. of days to produce effects	Hb p.c.		Tissue $CO_2$ -tensions, mm. Hg			
			Before exp.	After exp.	Skin		Abd. cav.	
					Before exp.	After exp.	Before exp.	After exp.
18 (died)	Hæmolytic	2	74	15	45	30	42	22
17 (died)	"	2	70	42	50	39	46	34
20 (died)	"	2	55	32	51	40	47	38
25	"	5	72	26	53	52	54	50
26	"	5	63	20	51	42	51	41
22	"	5	53	31	55	51	51	50
23	"	7	46	27	49	44	48	45
Av. for survivors			60	28	52	47	51	46
25 (died)	Bleeding	2	76	35	54	49	51	45
38	"	3	74	20	49	46	47	38
34 (S)	"	4	67	23	53	49	54	42
37	"	4	67	17	53	43	52	39
26	"	2	63	25	50	46	49	42
Av. for survivors			68	21	51	46	50	40
26	Injection of blood	6	63	102	47	43	47	46
25	"	5	69	112	50	47	52	51
32 (S)	"	5	68	106	51	46	56	48
Av. for survivors			67	107	49	45	51	48

(S) = splenectomised.

TABLE XI B. Tissue  $\text{CO}_2$ -tension as affected by increase (and decrease) of Hb p.c. due to previous prolonged exposure to low (and high)  $\text{O}_2$ -pressure in the inspired air; animal now breathing normal air.

Rabbit Ref. No.	No. of days exposure	Hb p.c.		Tissue CO <sub>2</sub> -tensions in mm. Hg			
		Before exposure	Day after termina- tion of exposure	Skin		Abd. cav.	
				Before exposure	Day after termina- tion of exposure	Before exposure	Day after termina- tion of exposure
Previously exposed to low O <sub>2</sub> -pressure:							
2	43	90	120	47	35	46	35
3	11	85	100	38	33	39	34
12	33	70	120	49	43	48	43
13	33	70	105	50	46	52	46
14	33	70	105	47	42	—	45
15	33	90	110	53	49	52	49
16	33	82	110	50	41	47	42
	Av.	79	110	48	41	47	42
Previously exposed to high O <sub>2</sub> -pressure:							
2	28	90	60	46	40	44	37
3	38	85	55	38	33	39	30
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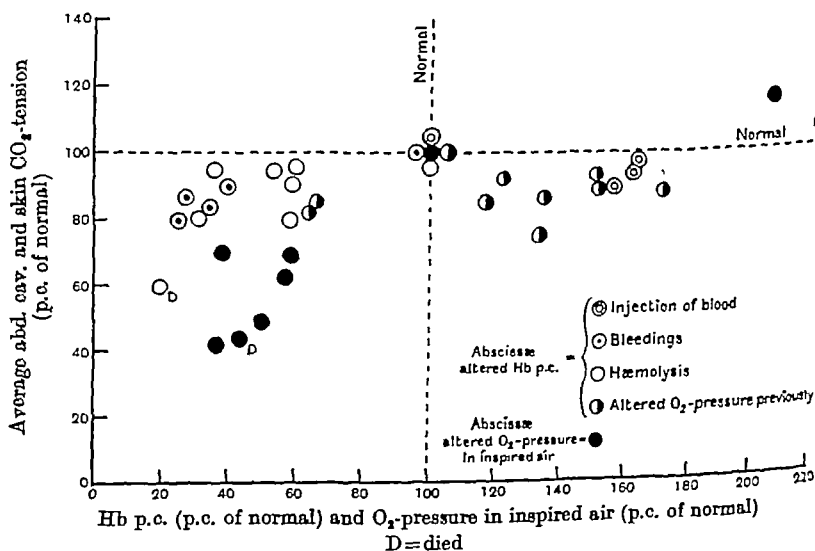


Fig. 4. Composite chart, showing (1) relations between hæmoglobin percentage (p.c. of normal) and  $\text{CO}_2$ -tensions in the tissues (p.c. of normal); and (2) relations between  $\text{O}_2$ -pressure in the inspired air (p.c. of normal) and  $\text{CO}_2$ -tensions in the tissues (p.c. of normal).

in Fig. 4. This figure has been arranged similarly to Figs. 2 and 3. Decrease of hæmoglobin percentage produced in some cases a definite

decrease in tissue  $\text{CO}_2$ -tensions, but in others there was not much effect. In some animals the respiratory centre was probably not so sensitive to  $\text{O}_2$ -deficiency as in others. In addition, any tendency towards fall of  $\text{CO}_2$ -tension in the tissues due to increased breathing might be obscured by an opposite effect such as caused by decrease in  $\text{CO}_2$ -carrying power of the blood due to loss of hæmoglobin. The decrease in  $\text{CO}_2$ -tensions in the tissues whilst breathing  $\text{O}_2$  at low pressure in the inspired air was much more marked than that produced by decreased hæmoglobin percentage; in the breathing experiments, of course, the hæmoglobin was normal or increased. We must remember also that hæmoglobin acts as an acid. Perhaps this acid-like action of hæmoglobin was demonstrated in the experiments in which hæmoglobin percentage was increased by injection of blood and by previous exposure to low  $\text{O}_2$ -pressure (Tables XI B and Fig. 4); here the  $\text{CO}_2$ -tensions in the tissues were below normal, whereas with animals with normal hæmoglobin percentage when breathing  $\text{O}_2$  at higher pressure than normal the  $\text{CO}_2$ -tensions were increased slightly or not changed at all (Fig. 4).

#### SUMMARY.

1. Tissue  $\text{O}_2$  and  $\text{CO}_2$ -tensions as affected by marked alterations of hæmoglobin percentage have been measured. The normal tensions are much the same in different rabbits, although their hæmoglobin percentage may differ markedly.

2. Marked decreases of hæmoglobin percentage in rabbits produced by injections of hæmolytic serum, by bleeding etc., markedly lowered the tissue  $\text{O}_2$ -tensions; the results indicate the possible degrees of  $\text{O}_2$ -deficiency produced in the tissues by anæmias. It is not possible to reduce the tissue  $\text{O}_2$ -tension to nil during life, even by extreme procedures. There is no evidence in favour of secretion of  $\text{O}_2$  by the lungs during breathing of  $\text{O}_2$  at low pressure, judging from the comparative effects of lowered hæmoglobin percentage and lowered  $\text{O}_2$ -pressure in the air upon tissue  $\text{O}_2$ -tensions.

3. Marked increases of hæmoglobin percentage produced by injection of blood and by previous exposure to low  $\text{O}_2$ -pressure in the air, definitely increased the tissue  $\text{O}_2$ -tensions. The improvement in  $\text{O}_2$ -tension is similar in degree to that produced by a short spell of vigorous exercise or a fit of convulsions.

4. Owing to oxygen deficiency tissue  $\text{CO}_2$ -tensions were decreased by bleeding and by hæmolysis, but to a less degree than when breathing  $\text{O}_2$  at low pressure in the air. During increase of hæmoglobin percentage,



the CO<sub>2</sub>-tensions in the tissues were also decreased, probably because hæmoglobin acts as an acid.

5. Reticulated red cells were increased from the normal figure of 30 to a maximum of 500 per 1000 red cells by bleeding and hæmolysis; whilst by increased hæmoglobin percentage, due to injection of blood, reticulated red cells were decreased from 30 to about 5 per 1000 red cells.

6. Splenectomised rabbits respond like some normal rabbits to hæmolysis, excessive bleeding and to injection of blood. Tested in this way the spleen does not appear to be of any great importance for maintenance of normal tissue O<sub>2</sub>-tension. Blood regeneration and blood destruction do not appear to be materially affected by removal of the spleen.

In addition to those mentioned in the text, I am indebted to Dr Leonard Hill and R. H. Davis, Esq., for granting facilities in the performance of the above experiments; and to Dr P. Hartley and Dr W. Purdy for assistance with some details of technique.

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## THE ACTION OF LIGHT ON THE EYE.

### Part III. The interaction of retinal neurones.

BY E. D. ADRIAN AND RACHEL MATTHEWS.

*(From the Physiological Laboratory, Cambridge.)*

THE optic nerve is unlike any other sensory nerve in that the receptor elements in the retina are not in immediate connection with the fibres of the nerve but are linked to them through a chain of neurones and synapses. This arrangement can hardly fail to affect the nature of the optic nerve discharge and a knowledge of how it is affected, besides telling us something about the eye, might also give results of more general interest concerning the action of the synaptic junction. In previous papers<sup>(1)</sup> on the action currents in the eel's optic nerve we have called attention to two features of the nerve discharge which might depend on synaptic conduction, namely, the long and variable reaction time and its dependence on the area illuminated as well as on the intensity of the light. In discussing these points we suggested a temporal and spatial summation in the nervous paths as a possible explanation, but we could not exclude the alternative possibility that they were due to the preliminary changes caused by the light before any nervous excitation had taken place. Meanwhile a chance observation had given us evidence of synaptic activity of an entirely different kind and the investigation of this has now led to the conclusion that the reaction time is also dependent upon synaptic activity.

#### *The response to even illumination of the whole retina.*

The observation referred to was made when we were examining the effects of moving a shadow across a large illuminated field. As a rule the shadow always occupied some portion of the field and the discharge in the optic nerve had the usual rapid and irregular succession of action currents. On one occasion, however, the entire field was evenly illuminated, and we noticed that when the light was turned out the "off" discharge showed a rhythmic succession of large waves with a frequency starting at 25 a sec. and falling gradually to about 6 a sec. (Fig. 2 A). We thought at first that the effect was due to light adaptation, for the

eye had been exposed to the light for several minutes, but in preparations arranged so that only a part of the retina was illuminated the most prolonged exposure gave nothing but the usual form of discharge. Eventually we found that the essential condition for obtaining a discharge of the rhythmic type is an even illumination of a very large part of the retina.

The arrangement we finally adopted is shown in Fig. 1. The eye is placed inside a hemispherical screen of opal glass (made by breaking off the top of an opal electric light bulb) and the screen is lit from behind by a 100 cp. lamp at a distance of 20–80 cm. Electrodes on the optic nerve lead to the 3-valve amplifier previously described and the amplified action currents are recorded with the capillary electrometer. With such

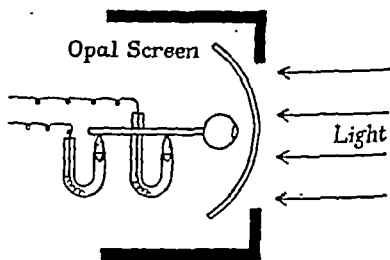


Fig. 1.

an arrangement most preparations of the eel's eye will sooner or later give the rhythmic type of discharge. As a rule it appears during the illumination but it is sometimes present in the "off" discharge as well, as it was in the original observation. The usual tests have been made to exclude mechanical and electrical artefacts and we are satisfied that the rhythmic oscillations in the record are an integral part of the optic nerve discharge.

Typical records are shown in Fig. 2. As a rule the rhythm does not appear until the light has been on for a few seconds. In the early stages of the response we have found occasional small patches of rhythm as high as 25 a sec. but as a rule the highest frequency at which it is fully developed is about 15 a sec. It falls gradually during the exposure and after 2 minutes it is usually about 5–6 a sec. This appears to be the lower limit; with a very long exposure the rhythm remains at this value for several minutes, but the waves become smaller and smaller and eventually disappear. The dependence of the rhythm on the duration of the exposure is shown in Fig. 2 E, F and G.

The other factor affecting the rhythm is the intensity of the light. In the middle of the record in Fig. 2 D the intensity was increased to about four times its previous value by moving the lamp nearer to the screen and the result is a quickening of the rhythm from 7.8 to 10.5 a sec. In some preparations the rhythm may be distorted by periodic beats or by short intervals of complete irregularity (Fig. 2 c).

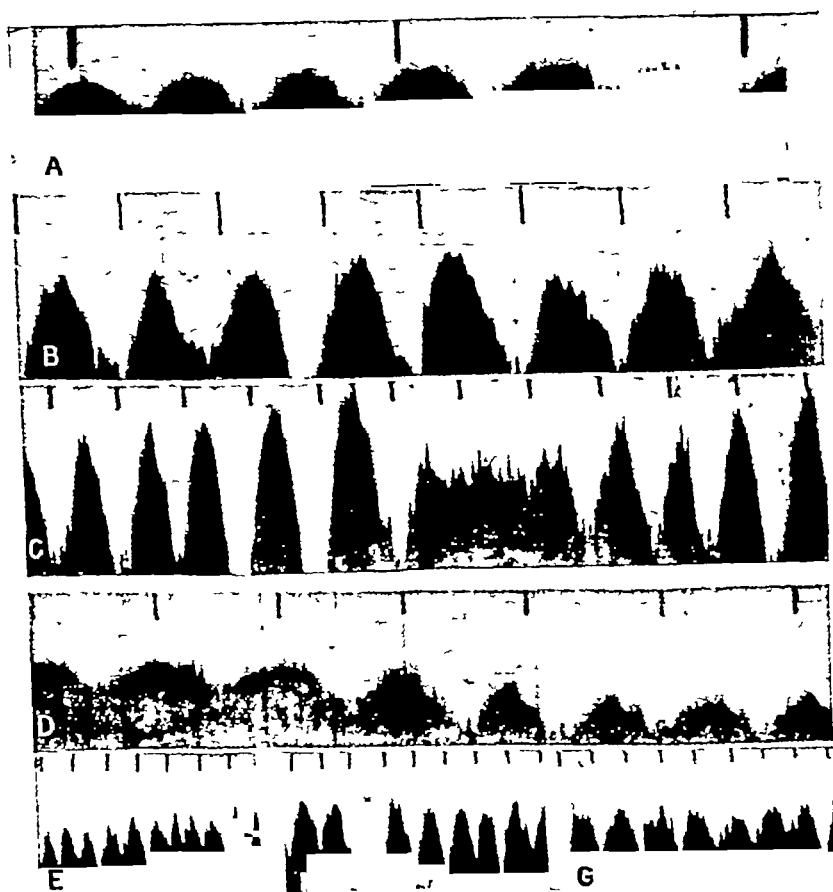


Fig 2. *Rhythmic action current discharge in cat's optic nerve with steady illumination of entire retina. Capillary electrometer and 3-valve amplifier. Black lines on records mark intervals of 0.125 sec*

- A. Exp. 1. "Off" discharge. Eye in darkness for  $\frac{1}{2}$  sec. after long exposure. Frequency 24 a sec.
- B. Exp. 10. 45 sec. after beginning of exposure. Frequency 8 a sec.
- C. Exp. 7. 40 sec. after beginning of exposure. Frequency 8 a sec. Regular beat in the rhythm.
- D. Exp. 2. Increase in frequency when light is increased. Illumination for 40 sec. Frequency 7.8 a sec. Half-way through the record the light is moved nearer the screen and the frequency rises to 10.5 a sec.
- E, F, G Exp. 7. Decline in frequency as exposure continues.
- |    |                    |           |             |
|----|--------------------|-----------|-------------|
| E. | 4 sec. from start, | frequency | 12.5 a sec. |
| F. | 35                 | "         | 8 "         |
| G. | 80                 | "         | 7 "         |

*Fröhlich's results.* Before discussing the cause of the rhythm we should draw attention to the likeness between our records and those obtained by Fröhlich(2) in the cephalopod eye. Fröhlich found that the currents led off from the eyeball often show rhythmic oscillations superimposed on the slower retinal currents of the usual type. In our former experiments with the illumination confined to a small part of the retina we were never able to detect anything of the kind either in records from the optic nerve or from the eyeball, but the rhythmic nerve discharge with the whole retina illuminated is so much like Fröhlich's effect that there can be no doubt of their common origin. The rhythms in the cephalopod eye are higher, for they are usually in the region of 30 a sec. and may reach as much as 90, and even illumination of the whole retina does not seem to be a necessary condition for their appearance; but in both eyes the frequency depends on the intensity of the light and declines gradually as the exposure is continued.

*The nature of the rhythmic discharge.*

If a record such as that in Fig. 2 *B* is examined it will be seen that there are a number of small oscillations at a much higher frequency which are apparently superimposed on the large regular waves. The small oscillations resemble those seen in an ordinary discharge with restricted illumination of the retina, and an analysis of the records made on rapidly moving plates shows that the whole discharge is really built up of these small oscillations. There is no gradual rise and fall of potential corresponding to the large waves, and the effect is merely due to the slow return of the mercury to the base line after a sudden displacement. Fig. 3 gives analyses of the large waves from two preparations and it will be seen that the rise in the electrometer record is due to the great increase in the number of the rapid potential changes which represent the action currents in the optic nerve fibres. During the period of decline these are few and far between, during the rise there are so many that the overlapping makes it impossible to count the number. The structure of the waves can also be seen from Fig. 2 *c* where a large wave is missed and a number of small oscillations take its place.

It is clear then that the rhythmic discharge is due to a more or less synchronous activity in a large number of the optic nerve fibres. The different ganglion cells have given up their usual independent fire of impulses and have taken to firing volleys. The fact that the impulses which make up the volley do not all appear at the same moment may be due to lack of complete co-ordination or it may be that each cell dis-

charges several times in rapid succession during the phase of activity. But it is clear that the majority of the ganglion cells are working more or less in unison with alternating periods of activity and rest.

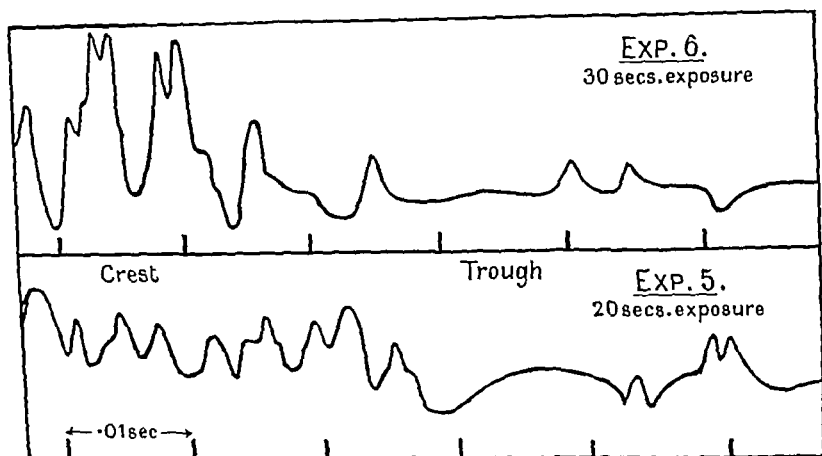


Fig. 3.

This state of affairs implies either a rhythmic stimulation of the sensory elements such as would be caused by a flickering light, or the rhythm must be determined by the nervous apparatus and there must be a close connection between the different ganglion cells. Fröhlich considers that the rhythmic discharge of the cephalopod eye is due to the rhythmic nature of the excitation process, i.e. that it is due to the fundamental properties of the nervous mechanism and not to some peculiarity of the antecedent photochemical change. He states that the occasional irregularities in the rhythm are probably due to the different parts of the retina failing to act synchronously, but he does not discuss the nature of the synchronising process. It seemed most probable to us that our records were dependent in the same way on the natural tendency to rhythmic discharge which is found in so many receptors and that the synchronous activity of the different neurones was due to nervous inter-connection in the synaptic layers of the retina, but we felt bound to consider also the possibility of a rhythmic stimulation. The illumination of the entire retina for 10 sec. or more might exhaust the reserve of photosensitive material and it was conceivable that a state of affairs might result in which decomposition could only occur in intermittent periods separated by intervals of rest. The effect would then be much the same as that due to a flickering light.

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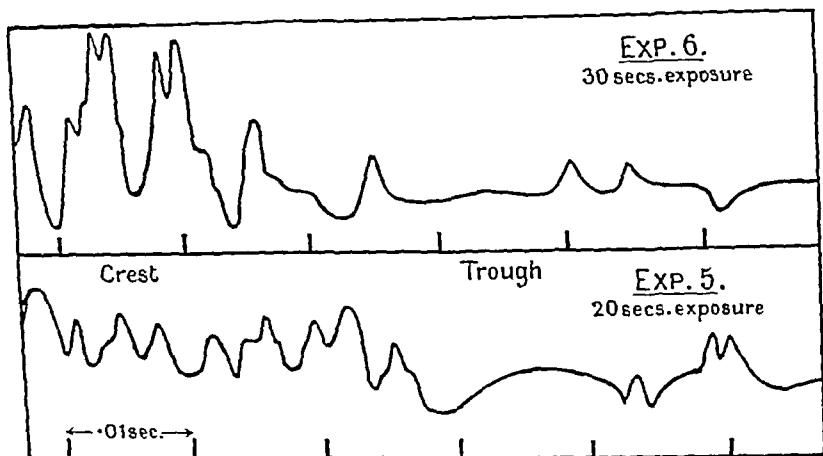


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*The response to flicker.*

As we had no records of the optic nerve discharge to flicker, we arranged the optical system so that the beam of light from the lamp

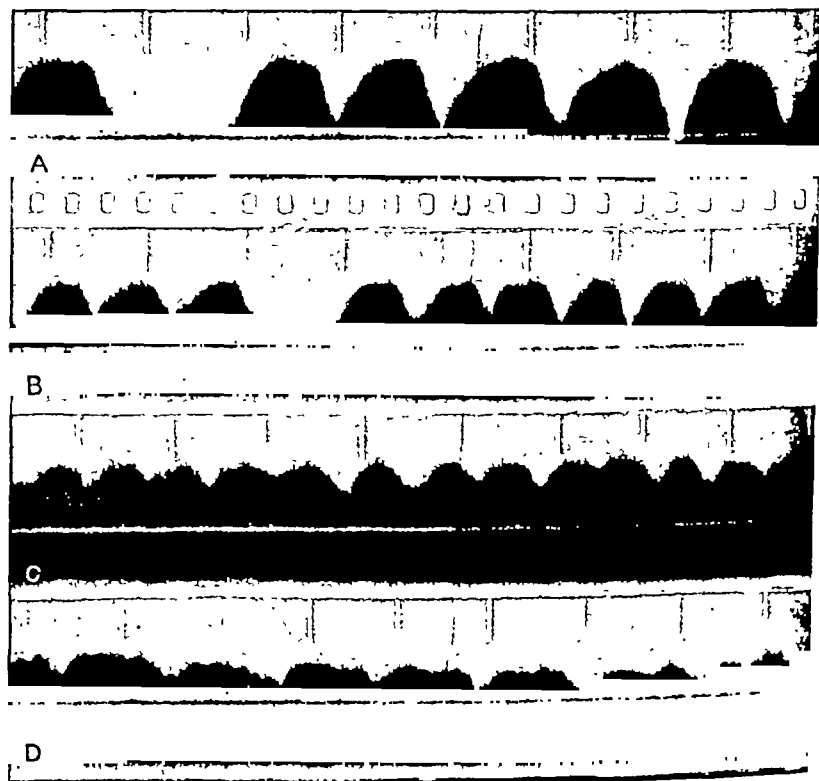


Fig. 4. *Rhythmic discharge to flicker. Exp. 15. Whole eye evenly illuminated.*

A.	Rate of flicker	7 a sec.	Response at same frequency.
B.	"	10 "	" "
C.	"	12 "	" "
D.	"	14 "	" 7 a sec.

Time marker gives intervals of 0.125 sec. The horizontal white line in Fig. 4 and Fig. 7 is made by a pointer attached to the minute hand of a watch. It moves across the camera slit at 2.5 mm. in 10 sec. and shows the duration of the exposure at any moment. The rate of flicker is signalled by the faint bands of shadow at the foot of each record.

could be interrupted periodically by a revolving disc with apertures cut in its rim. The light was brought to a point focus in the plane of the disc and the periphery was divided into 16 equal segments of which

8 were cut away so that the periods of light and darkness were equal. The disc was 2 ft. in diam. and the spindle ran in ball bearings. It was rotated by hand, and the speed at any moment was signalled on the record by mounting another lamp behind the disc and allowing the flashes from it to fall on one side of the camera slit. A series of records with different rates of flicker is given in Fig. 4. In these records the whole retina was evenly illuminated. When the rate of flicker is 13 a sec. or less, the optic nerve discharge shows a series of large waves at the same frequency. With flicker just above 14 a sec. there is some indication of a rhythmic discharge at half the rate, but a flicker of 15 a sec. gives a discharge of the usual irregular type with no periodic waxing and waning in intensity.

The fusion point depends both on the intensity of the light and on the size of the retinal image, as it is known to do for the visual sensation in man. For instance, in the preparation which gave the records in Fig. 4 the fusion point fell to 10 a sec. when the area of retina illuminated was reduced to a disc of 0.67 mm. diam. and to 7 a sec. when the disc was 0.17 mm. diam. In another preparation with an area of 0.67 diam. and the same light, the fusion point was just over 11 a sec. and it fell to 5.5 a sec. when the intensity of the light was reduced to one-sixth of its previous value.

No doubt an intense light covering the whole retina would give a fusion point somewhat higher than 15 a sec. but it is a striking fact that the limiting rate of the rhythmical discharge with flicker is of the same order as the limiting rates we have observed with the steady light. The form of the large waves is not quite the same, for there is a sharper rise and more gradual fall with the flicker, but this is less evident at the higher rates. If we grant, then, the rather unlikely possibility that a continuous illumination of a large area may give rise to an intermittent stimulating effect, the likeness of the records might certainly be taken to support this explanation. Fortunately we have been able to dispose of this possibility by experiments on the effects of strychnine in promoting the rhythmic type of discharge.

#### *Effects of strychnine.*

When a freshly dissected eye is exposed to even illumination over the whole retina, the rhythmic response in the nerve is often absent altogether. After several periods of illumination lasting about a minute and separated by intervals of about 5 minutes, indications of a rhythm begin to appear in the record after the light has been in action for 30 sec. or

more. In subsequent exposures the rhythm becomes more clearly marked, it appears sooner after the light is turned on and its period is then higher than in the later stages of the earlier records. Mere lapse of time without stimulation does not appear to favour the development of the rhythm, nor does stimulation for very long periods with very short intervals of rest, but it is difficult to make sure of this as there is a wide range of variation between different preparations. The delay in the appearance of the rhythm was much greater in experiments made in November and December than in May and June, and in some of the former we were never able to establish a definite rhythm.

Now the chief effect of strychnine is to favour the processes of conduction through the synapses of the central nervous system. Not only does it produce a condition where the slightest stimulus to any receptor will cause reflex activity in all the muscles of the body, but it may also produce synchronous rhythmic discharges from all the motor neurones over a wide area of the spinal cord. Records of the action currents from muscles during a strychnine spasm may reveal a wide variety of discharge, ranging from the rapid, irregular oscillation like that of the normal reflex electromyogram to rhythmic discharges at periods ranging from 70 to 2-3 a sec. The slower types of rhythmic discharge are often multiple, and such multiple waves nearly always appear with large doses. Fig. 5 shows some typical electromyograms under strychnine made with the string galvanometer by one of us (E. D. A.) working with Miss Sybil Cooper in 1924. In Fig. 5 E and F simultaneous records were made from two muscles and it will be seen that the discharges in the two are synchronous. In one of these the sensory roots from the muscle had been cut, so that the rhythm cannot have been determined by stimulation of the receptors of this muscle, but must have spread to its motor neurones from other parts of the central nervous system. It is clear too from Miss Buchanan's experiments (3) that the rhythms, or at least the slower types of rhythm, are centrally and not peripherally determined, for they vary with the temperature of the cord and not with that of the muscles. When they appear in the spinal preparation they cannot be due to a discharge from a single controlling centre in the brain, and we must imagine that a large number of the neurones in the cord have become linked together and are working more or less in unison.

Strychnine, then, might be expected to favour the development of rhythmic discharges in the optic nerve if these are due to the interaction of the neurones in the retina, and there is no reason at all why it should

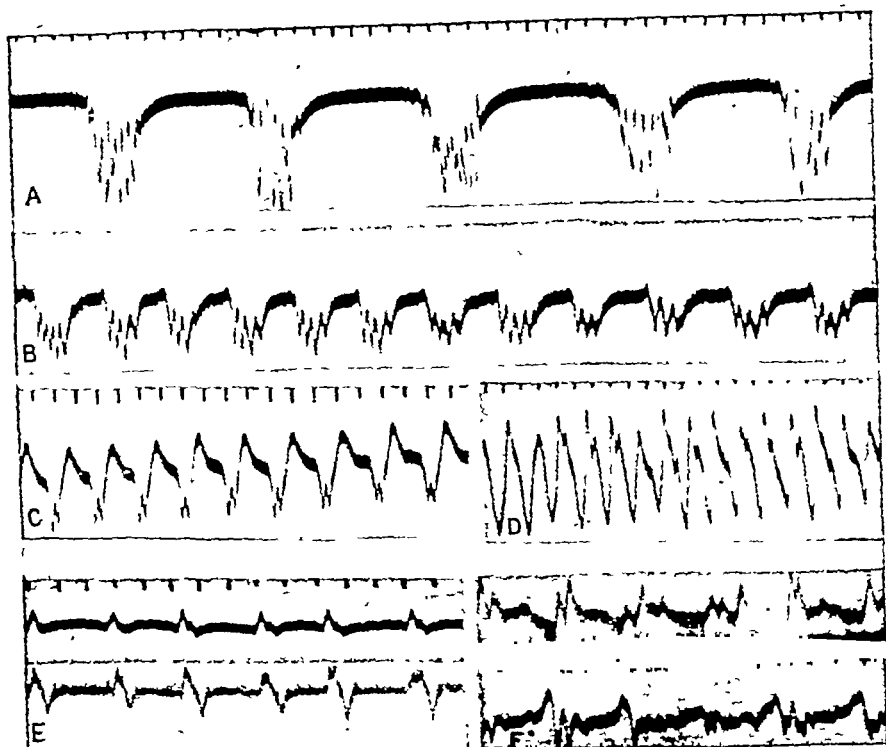


Fig. 5. Rhythmic discharges from the spinal cord induced by strychnine. String galvanometer,  $4\mu$  string. Resistance  $2500\omega$ . Magnification 440. String just aperiodic. Maximum deflection reached in 0.006 sec. In E and F the second string (lower shadow in E) had a resistance of  $4300\omega$  and a diam. of  $4.5\mu$ . Time marker gives 0.02 sec. intervals.

A, B, C and D. Spinal frogs (four different preparations). Leads from gastrocnemius. Samples of rhythmic electromyogram during strychnine spasm.

E and F. Simultaneous records from two muscles in spinal cats during strychnine spasm.

E. Upper string to left tibialis anticus (posterior roots divided); lower string to right tibialis anticus (nerve supply intact).

F. Another animal. Upper string to right tibialis anticus; lower string to right quadriceps.

NOTE.—Needle electrodes were used for leading off in the records shown in E and F. Forbes<sup>(10)</sup> has criticised their use on the ground that a pair of electrodes in one muscle may be affected by action currents in another muscle. In these experiments, however, we found that action currents in the hamstring muscles had only a very slight effect on electrodes in the quadriceps of the same leg (spinal cat) and none on electrodes in the tibialis anticus. Thus the synchronous movement of the two strings cannot be attributed to current spread.

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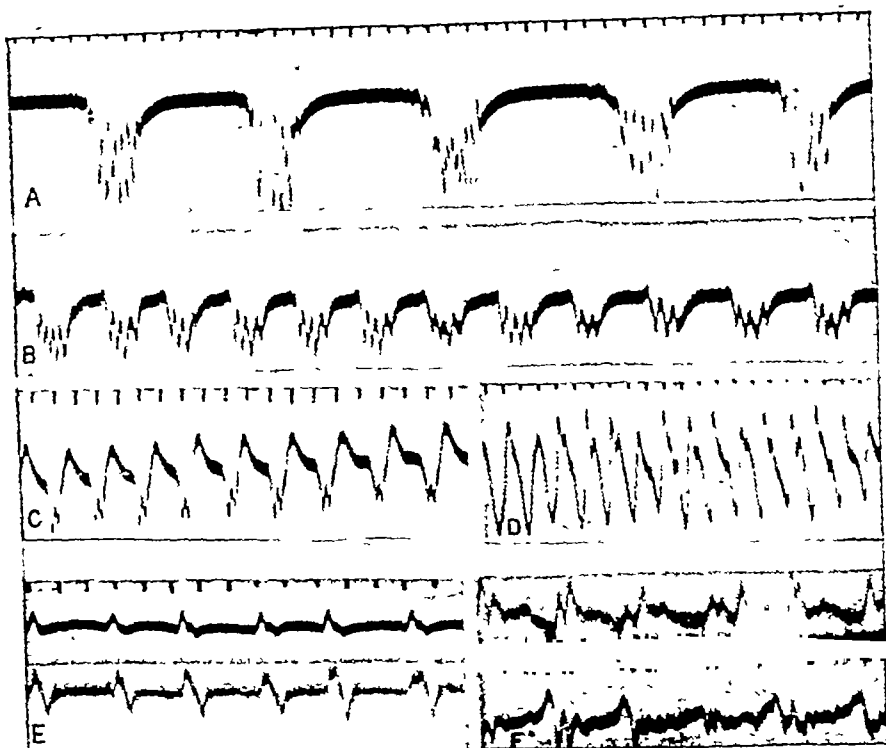


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have any effect if the rhythmic discharge is due to a regular waxing and waning of the preliminary photochemical changes.

Apart from a single experiment in the summer, all our observations with strychnine were made in the winter months when the rhythmic discharge took a long time to appear in the normal eye. To simplify the application of the drug to the retina, the front half of the eyeball was cut away and some of the vitreous humour removed. The posterior half of the eyeball was supported in a cup-shaped vulcanite holder with the nerve hanging down through a slit in the holder and making contact with the two leading off electrodes. Light from an opal glass disc was thrown on to the retina by a small mirror fixed at  $45^\circ$  above it (Fig. 6).

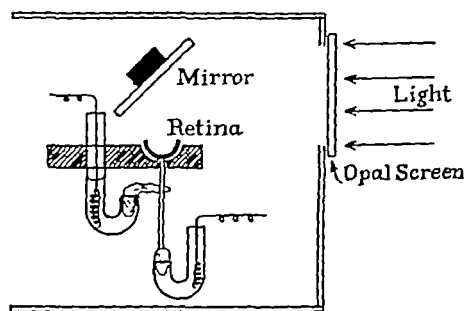


Fig. 6.

The removal of the front half of the eyeball does not interfere with the working of the retina, as judged by the magnitude and reaction time of the optic nerve discharge, but in these preparations we were seldom able to produce more than a suggestion of a rhythmic discharge without strychnine.

A drop of 0.01 p.c. strychnine nitrate in Ringer gives an obvious effect within a few minutes. Although the eye remains in darkness the optic nerve record begins to show large rhythmic fluctuations at a frequency which is usually between 2 or 4 a sec. Records of this "resting" discharge are given in Fig. 7 A and B, and the general likeness to the string galvanometer records in Fig. 5 is apparent at once. When the light is turned on there is a very large discharge of impulses and the rhythm usually disappears, to return again as soon as the light is cut off. At a later stage in the action of the drug there is sometimes a more rapid "resting" discharge (6-8 a sec.) and on illumination after a short period of irregularity the discharge becomes rhythmic again at a higher frequency (Fig. 7 C and D). In some preparations with small doses the

resting rhythm does not appear, but we find instead a rhythmic response during illumination which has all the features of that seen in the normal eye, the rhythm beginning after about 5 sec. with a frequency of 15-12

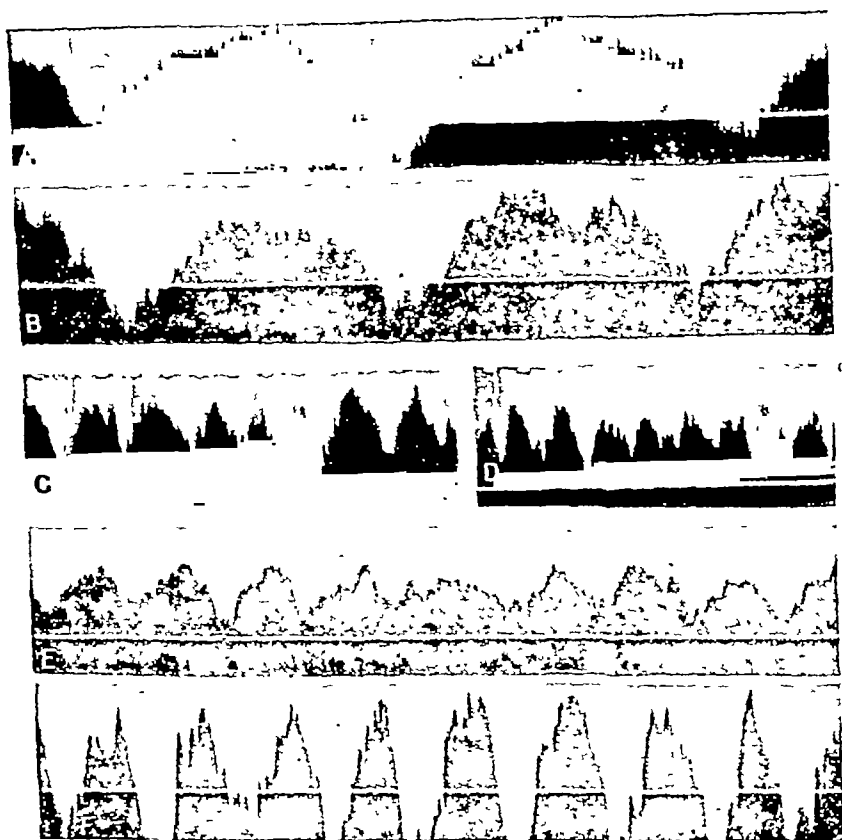


Fig. 7. Rhythmic discharges from the retina induced by strychnine.

- A. Eye in darkness. Exp. 17. 90 sec. after application of 0.01 p.c. strychnine. Resting discharge with a frequency of 2 a sec.
  - B. Eye in darkness. Exp. 19. 5 min. after strychnine. Resting discharge with a frequency of 3.3 a sec.
  - C and D. Exp. 19. 8 min. after strychnine.
  - C. Eye in darkness. Resting discharge at 8 a sec.
  - D. In light for 5 sec. Discharge at 13 a sec.
  - E and F. Rhythmic discharge during steady illumination.
  - E. Exp. 17. 5 min. after strychnine. Light on for 12 sec. Frequency 7 a sec.
  - F. Exp. 19. 20 min. after strychnine. Light on for 15 sec. Frequency 8 a sec.
- Time marker gives intervals of 0.125 sec. in all records.



a sec. and declining gradually to 6 a sec. or less (Fig. 7 E and F). We have found occasional patches of regular rhythm between 20 and 30 a sec. in the initial stages of the discharge, but these are never maintained. Thus the rhythms developed under strychnine have an upper limit which agrees with that found in the normal eye.

Strychnine, then, will produce very slow rhythmic discharges from the optic nerve closely resembling the discharge from the spinal cord and it will also produce the more rapid rhythm during illumination. There can be no doubt that both types of rhythm are due to the drug, for they appear within a few minutes of its application in preparations which can only be made to develop the rhythmic type of discharge with extreme difficulty in the absence of strychnine. We may be fairly certain, therefore, that the rhythmic discharge is due to the establishment of a nervous connection between the different ganglion cells of the retina, a connection of the same kind as that established under strychnine between the different motor neurones of the cord.

#### *Interaction of distant areas.*

These conclusions show that the retinal neurones in the eel's eye may become so closely coupled that they work in unison. It follows that the neurones leading from adjacent points on the retina may have a considerable effect on one another's activity, and we may enquire whether a nervous interaction of this kind will not also account for the relation between the reaction time and the size of the illuminated area. This relation has been studied in a large number of experiments, all of which have shown that the reaction time, *i.e.* the interval between the beginning of the illumination and the beginning of the optic nerve discharge, is much shorter when the illuminated area is large. As a first approximation it appears that with areas not exceeding about 1 mm. diam. on the retina the same change in reaction time is produced by doubling the area as by doubling the intensity of the light—in other words, the reaction time depends simply on the quantity of light reaching the eye, without regard to its distribution. This relation was discussed in Part II(4); it means that the effects of the light must be transmitted to some region whose extent is more or less independent of the extent of the illuminated area, and this might be done either by a widespread diffusion of the preliminary photochemical changes or by nervous transmission to a group of ganglion cells which are equally in touch with all parts of the area. In the discussion it was assumed that the ganglion cells were independent, but that each was connected with retinal elements

scattered over a wide area; the possibility of a nervous connection between different ganglion cells was not considered, but it is clear that the same kind of summation might take place whether we have to deal with a single ganglion cell activated by a number of elements scattered over the retina, or with a group of connected ganglion cells activated by the same elements.

We have seen that a widespread connection may be formed between the different ganglion cells in the normal retina and that the occurrence of this connection is favoured by strychnine. Strychnine, therefore, might be expected to favour the summation effect and to increase the range over which it occurs, if that effect depends on the interaction of a large group of neurones. On the other hand, if it is due to a fixed structural arrangement such as that considered in Part II, there is no reason why strychnine should have any influence on it.

To test the point it is necessary to illuminate two or more small areas on the retina separated by such a distance that there is normally no summation between the two, *i.e.* no shortening of the reaction time when the two are illuminated simultaneously instead of one at a time, and then to see if any summation is produced with a small dose of strychnine. The intensity of the light must be adjusted so that the reaction time for the single area is moderately long, for if it approaches the minimal value the effects of summation would be inappreciable. Preliminary experiments showed that four small areas (about 0.22 mm. diam.) set at equal distance on a circle of 1 mm. diam. on the retina would give a shorter reaction time when all are illuminated together than when each is illuminated alone, but that there was usually no shortening when the diam. of the circle was raised to 2-3 mm. The arrangement of the apparatus is shown in Fig. 8. The front half of the eye is cut away as in the other experiments with strychnine and the retina and optic nerve are set up in the same holder. A 1-inch microscope objective is held vertically above the retina and can be focussed by rack and pinion. A mirror at 45° just above the objective reflects into it the image of four holes in a metal screen which is placed just in front of a piece of ground glass lit from behind by a 100 or 500 c.p. lamp. A metal disc with an aperture in its rim can be fixed to the centre of the screen so that each hole can be exposed singly and the size of the holes and the distance of the screen from the eye is adjusted so that the image focussed on the retina consists of four bright spots, each 0.22 mm. diam. set at equal distances on the circumference of a circle 2 mm. in diameter (the distance between adjacent holes being 1.4 mm.). The

retina was usually arranged so that the entrance of the optic nerve lay in the centre of the circle with the four bright spots round it. In eels

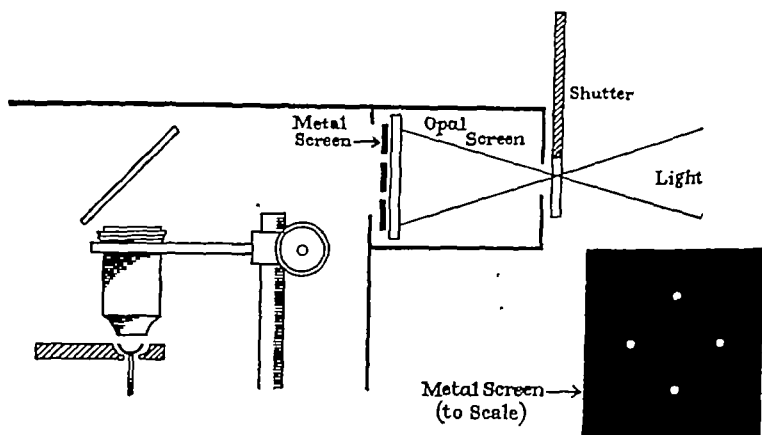


Fig. 8.

of the size we were using (about 2 ft. long) the average diameter of the retina is about 7-9 mm. and the distance between the bright spots is such that the two opposite one another would subtend a visual angle (in water) of about  $30^\circ$  with the eyeball intact.

The procedure was as follows. The retina was first exposed to all four bright areas simultaneously, then to each one in turn and then again to all four together and the records were developed to see if any summation had occurred. As a rule the magnitude and reaction time of the optic nerve discharge varied considerably for the different areas taken singly and often one of the four would give a very long reaction time and a very small discharge, but there was rarely any clear difference between the most rapid reaction time from a single area and the reaction time with all four together (although the magnitude of the discharge was naturally greater). If the records were satisfactory on this point a drop of 0.01 or 0.005 p.c. strychnine solution was placed on the retina in exchange for the Ringer's fluid which covered it before. Within a few minutes the increased size of the discharge on illumination and the much greater resting discharge showed that the strychnine had begun to take effect, and very soon (within 5 minutes with 0.01 p.c. strychnine) the slow rhythmic waves appeared with the eye in darkness. The exposures were then made as before and the records were repeated at intervals of several minutes.

The result of this experiment can be seen from Table I which gives the reaction times before and after strychnine for three experiments,

TABLE I. Retina illuminated over four areas *A*, *B*, *C* and *D* arranged as in Fig. 8.

	Before strychnine.	After strychnine (0.01 p.c.).		
Area exposed	Nerve reaction time	Nerve reaction time (12 min. after application)		
Exp. 23.				
A	0.18 sec.	0.31 sec.		
B	0.21	0.32		
C	0.18	0.37		
D	0.18	0.29		
A+B+C+D	0.18	0.23		
Exp. 24.				
		(7 min. after application)	(22 min. after application)	
A	0.10 sec.	0.30 sec.	0.265 sec.	
B	0.16	0.30	0.245	
C	0.16	0.33	0.24	
D	0.13	0.32	0.265	
A+B+C+D	0.10	0.15	0.19	
Exp. 25				
		(2½ min. after application)	(12 min. after application)	(24 min. after application)
A	0.23 sec.	0.32 sec.	0.31 sec.	0.33 sec.
B	0.235	0.34	0.29	0.33
C	0.28	0.34	0.325	0.33
D	0.41	0.33	0.30	0.34
A+B+C+D	0.225	0.25	0.23	0.265
(3 mm. disc)	0.09)			

and from Fig. 9 which gives some of the records. Before strychnine the reaction time of the discharge with all four areas is the same as the fastest reaction time for a single area, but after it the reaction time for the four areas together is much shorter than for any one singly, *i.e.* the areas are no longer acting independently and the separate excitations are summated on their way to the optic nerve fibres.

In *Exp. 24* the reaction times before strychnine are so short that there would not be much opportunity for the summation effect to show itself, and although there is no doubt about the summation after strychnine we cannot be certain that it was absent before. In *Exps. 23* and *25*, however, the reaction times are well above the minimal value, and the summation would certainly appear if it were present to anything like the same extent as after strychnine. In a further series of experiments using screens with 2, 3 or 5 holes we have occasionally found summation at distances as great as 1.2 mm. in the normal eye, but after strychnine the range is so much increased that the reaction time for a

given quantity of light remains the same whether the light is concentrated into a single patch, or spread over four small areas on a circle of 4 mm. diameter, and our optical system has not allowed us to use any wider separation than this.

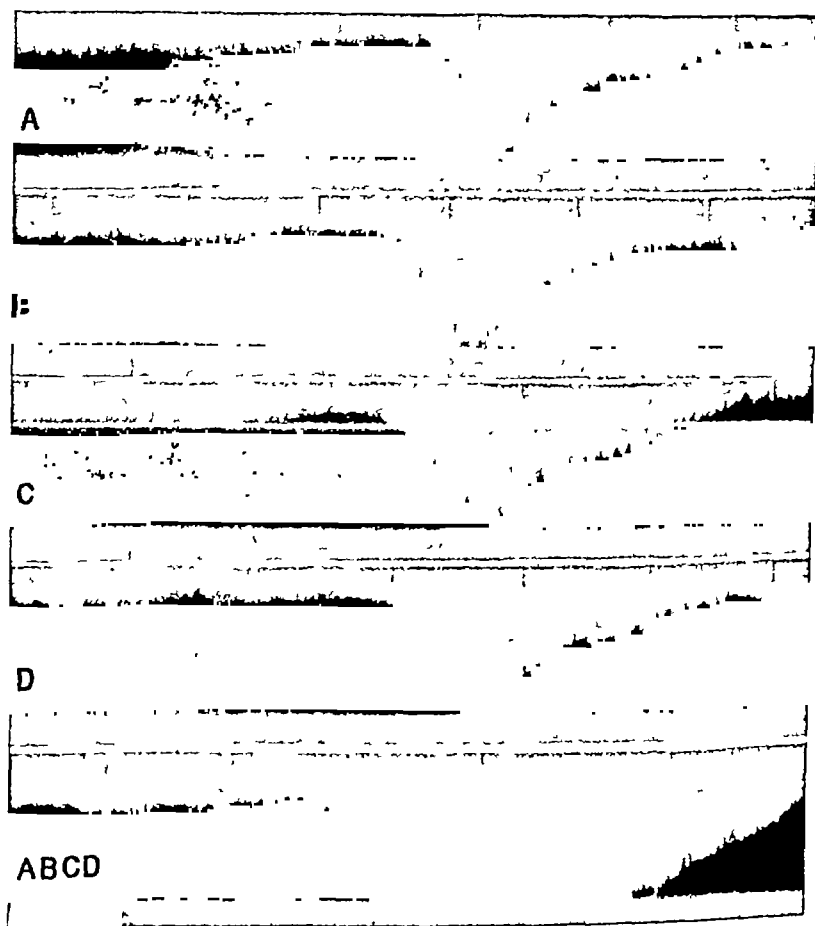


Fig. 9. *Interaction of four retinal areas after strychnine. Exp. 24. 22 min. after 0.01 p.c. strychnine. The slow rhythmic response in the dark has subsided. The electrometer is shunted to  $\frac{1}{2}$  normal sensitivity to show the form of the discharge.*

Time marker (above) gives 0.125 sec. intervals. Beginning of illumination marked by shadow at the foot of each strip.

A alone.	Reaction time 0.265 sec.	C alone.	Reaction time 0.24 sec.
B	" " 0.245 "	D	" " 0.265 "
A + B + C + D. Reaction time 0.19 sec			

As from sight in night seem what these results could be explained very easily on the view that scototopia produces a great increase in the sensitivity of the receptors for a spreading photochemical change which then spreads over a wider area. But although the discharge of impulses for a given stimulus is invariably greater after scototopia, we have found no evidence of a true increase in excitability. The threshold intensity of illumination cannot be measured accurately either before or after scototopia, but there is no obvious change in the quantity of light required to give a really definite response in the optic nerve when a single area of the retina is illuminated. Again, if scototopia acts by producing an increase in the stimulating power of a given light, we should expect the reaction time for a given light intensity to become shorter after scototopia. We find, however, that the whole curve relating reaction time to intensity is either unchanged or else there is a definite lengthening after scototopia.

There rises out the possibility that the scototopia effect is due to an increase in the sensitivity of the retina, and we may conclude that the main action of the drug is to produce a great extension of the lateral connections in the nervous layers. Such an extension will account for all the effects we have described—the development of the slow or first rhythm, the increased range of interaction and the greater magnitude of the impulse discharge in the optic nerve.

The fact that scototopia does not lead to any shortening of the reaction time for a single area is particularly interesting for it must mean that although it causes a more widespread activity in the synaptic layers it does not facilitate the transmission of this activity to the optic nerve fibres. The reaction time is not reduced and is dependent as before on the area and intensity of the light, thus although after scototopia the excitation of a small number of receptors may produce an activity of almost the same extent in the synaptic layers, the rate at which this activity develops is no faster than before and is still dependent on the number of receptors excited and on the intensity of the light. It fact of the neural properties being the same as those of the skin and muscles it seems most probable that the rate of the synaptic transmission is determined both before and after scototopia by the total number of impulses which reach the synaptic layers during the initial stages of the illumination.

#### DISCUSSION

It is clear that the extremely disorganized and the interaction of which depend on the nervous connections between the different

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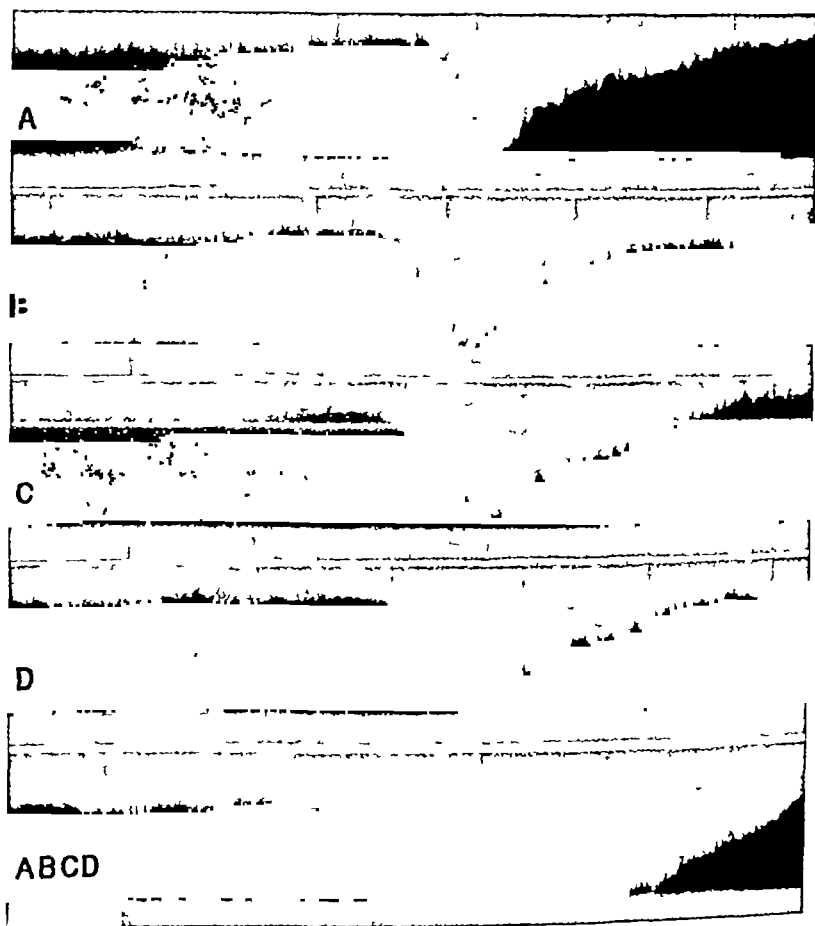


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time a dominant region forces the whole layer to work in unison with it. This could only occur if all parts of the layer tended to beat at the same rate, for a tendency to a much slower or faster rhythm in any part would sooner or later break through the dominant rhythm or would prevent it from developing. When a restricted area is illuminated the excitation will not be uniform except perhaps in the centre of the field, for it will probably spread with diminishing intensity into the surrounding parts of the synaptic layer. Thus a rhythmic discharge is not developed unless the whole retina is illuminated uniformly. We find, however, that some lack of uniformity will not interfere with the rhythm when once this is fully established. A narrow band of shadow thrown across the field will not cause much disturbance of the rhythm when this has already developed under an even illumination of the whole field, though the disturbance is much greater if the shadow is present from the beginning (see Fig. 10).



Fig. 10. Exp. 4.

- A. Eye exposed to bright field divided by a narrow band of shadow. The shadow was present throughout the exposure which had lasted 30 sec. before the record was made. There are indications of a rhythm interrupted by beats.
- B. Same eye exposed for 35 sec. to a uniform field.
- C. The same eye was exposed to a uniform field for 20 sec. and the field was then divided by a band of shadow. After a few seconds of irregularity the rhythm reappears, though it is not so well marked and a distinct beat is present.

It is noticeable that the rhythm does not appear until the light has been on for 5 sec. or more, that its frequency is then seldom greater than 15-20 per sec. and that it may be absent in the early stages of



elements of the retina, we must regard one or both of the synaptic layers as a sheet of nervous material capable of acting as a whole, not as a number of independent pathways leading from particular receptors to particular ganglion cells. The histology of the retina certainly supports this view for the elaborate branching processes of the retinal neurones (cf. Dogiel(5), Cajal(6)) and those of the amacrine and horizontal cells would give an ample structural basis for communication between the different regions.

When a restricted area of the retina is illuminated, there must be a widespread activation of the synaptic layers. After strychnine the illumination of a patch 0.2 mm. in diam. must cause an activation covering a circle at least 1.4 mm. in diam. In the normal eye the circle will be smaller, perhaps not more than 1 mm. in diam., but the spread of the excitation will enable many groups of retinal elements to affect the same ganglion cell and many ganglion cells to be affected by the same retinal element, so that anything like an exclusive connection between a given group of receptors and a given ganglion cell must be out of the question.

We have at present no evidence as to the kind of activity developed in the rods and cones. They may discharge a series of impulses like a peripheral end organ, or possibly they may remain continuously in the excited state, and this continuous excitation may be transmitted to the synaptic layers. But here at least we have evidence of the familiar type of intermittent activity, for the rhythmic nerve discharge from the ganglion cells must be accompanied by a rhythmic waxing and waning of the excitation in the synaptic layer which connects them.

The production of the rhythmic discharge is not surprising, for there are many instances where a number of rhythmically beating cells begin to act in unison as soon as a connection is established between them. A good example is that recorded by Lillie(7) where the spermatozoa of *Nereis* aggregate into a clump with the result that their tails begin to beat in unison; the appearance of one or more dominant rhythms in cultures of embryonic heart muscle(8) is another instance of the same kind. Presumably a group of several units which happen to be beating in unison comes ultimately to dominate the rest because the refractory state following each beat of the group interferes with the independent activity of the other units and forces them gradually into line.

In the same way we may suppose that at the start of the illumination the various parts of the synaptic layer in the retina are beating (*i.e.* becoming active and inactive) independently but that after a short

sterno-cutaneous organ gives a higher frequency, but the rate of adaptation to the stimulus may well be more rapid in the eye than in the

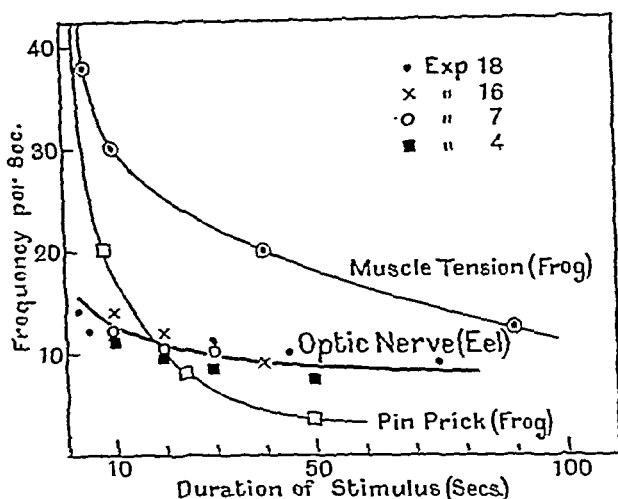


Fig. 11. Effect of continued stimulation on impulse frequency from peripheral end organs (frog) and on rhythm of optic nerve discharge (eel).

muscle receptor. for the latter becomes adapted more slowly than any other organ which has been studied. It is quite probable, therefore, that the rhythm of the optic nerve discharge is produced by the same kind of process as that operating in the peripheral sense organs<sup>1</sup>.

We cannot tell from the records how the impulses in each nerve fibre are spaced out. In the slow waves of the strychnine effect in the resting eye the period of activity is so long that each discharge of the individual ganglion cells is probably multiple, but in the more rapid rhythms each discharge may be single and the impulses in each nerve fibre may be evenly spaced as they are in the discharge from a peripheral end organ under a constant stimulus. If they still occur in groups it is very difficult to see how the brain can distinguish the discharge from that produced by a flickering light.

<sup>1</sup> Rhythmic discharges of low period are sometimes found when the central nervous system is subjected to continued excitatory and inhibitory stimuli at the same time, and Cooper and Denny-Brown (10) suggest that the clonus which follows cortical stimulation is due to an inhibitory effect which causes a periodic extinction of the concurrent excitation. It is possible that the development of the rhythmic discharge in the optic nerve may depend on the gradual building up of an inhibitory process in the retina, but the rhythm is much higher than those usually found in the discharges in question.

the action of strychnine although there is a slow rhythmic discharge in the dark. The explanation is evidently that the synchronous beat cannot occur until the rhythm of the different parts of the synaptic layer has fallen to 20 a sec. or less, for in the early stages of the discharge of the normal eye and in the enhanced activity caused by strychnine the excitation will be more intense and we should expect the rhythm to be much higher. If this is correct, the rhythmic discharge ought to appear sooner when the intensity of the light is less. It has done so in several preparations, though a certain minimal intensity seems to be necessary to maintain the discharge.

Several factors might prevent the development of a synchronous beat above a certain frequency. The connecting paths might recover too slowly to conduct a high rhythm throughout the synaptic layers, or possibly at the higher rates of response the frequency is less uniform in the different regions. The limiting rhythm with a flickering light must also depend on some property of the synaptic layers since the fusion point depends on the area illuminated. The two cases are not strictly comparable, but it is hard to resist the conclusion that a common factor limits the rhythm to 15-20 a sec. in both.

In the cephalopod eye Fröhlich found a rhythmic response of the retina at frequencies as high as 90 a sec. without the necessity for uniform illumination of the whole eye. Here, then, the connections must be more easily established than in the eel, though it is true that Fröhlich did not find the rhythmic response in every preparation. In the frog's optic nerve we have only once succeeded in finding a rhythmic response and then the rhythm was only maintained for a few seconds. The effect of strychnine on the frog's eye has not been investigated.

It seems probable that the rhythmic activity of the ganglion cells is determined by a rhythmic discharge of impulses from the receptor elements of the retina synchronised by the connections in the synaptic layers. The range of frequency of the rhythms we have observed is certainly low, but it is not outside the range of frequency of the impulse discharges from peripheral end organs in cold-blooded animals. In the end organs of the frog the maximum frequency is probably in the region of 100 a sec. but this declines more or less rapidly if the stimulus is continued and with weak stimuli acting for long periods discharges at 7 a sec. or less are often seen<sup>(9)</sup>. Fig. 11 gives several curves showing the lengthening of the rhythm of the optic nerve discharge compared with that of the end organs in the frog's sternocutaneous muscle and of the pain receptors in the frog's skin. The

central-nervous system gives a higher frequency, but the rate of adaptation to the stimulus may well be more rapid in the eye than in the

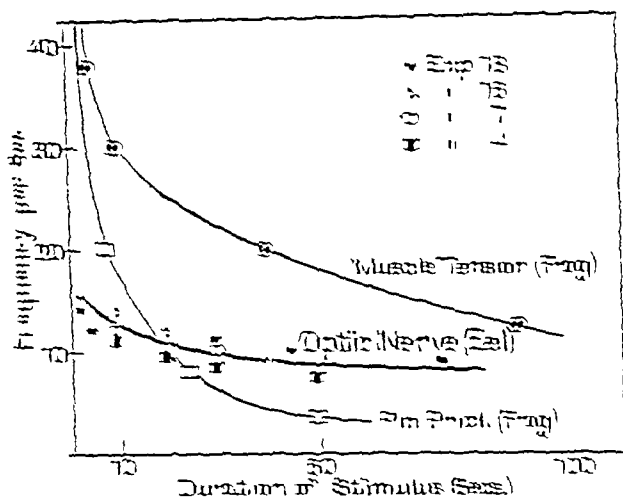


Fig. 13. Effect of continued stimulation on impulse frequency from peripheral nerve (frog) and on duration of optic nerve discharge (cat).

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rhythm might be comparable to that produced in the optic nerve by a flickering and not a steady light. Explanations on these lines have often been advanced to account for the rhythm in the electromyogram (12). There is one case, however, where the synchronisation must be due to interaction in the cord itself; this is the experiment made by Dusser de Barenne and Brevée (13) in which the dorsal part of the cord was treated with novocaine, and the electromyogram of the contraction due to touching the cord became more and more regular as the sensory neurones were put out of action. The electromyogram has been mentioned because in one respect it shows a remarkable likeness to the optic nerve discharge. With a feeble contraction there is no sign of the dominant 50 a sec. rhythm: to produce it the contraction must be powerful, i.e. a large number of motor neurones must be involved. To produce the rhythmic discharge in the optic nerve the whole retina must be illuminated and here too there is no sign of a dominant rhythm when only a few neurones are activated.

In the electromyogram we are concerned with a rhythm of 50 a sec. or more, but perhaps the most striking example of widespread rhythmical activity in the spinal cord is that occurring at a much lower frequency in the scratch reflex, where a large number of muscles contract together with a period of  $\frac{1}{4}$  a sec. In this case also the rhythm is determined within the spinal cord, for Sherrington (15) has shown that it is in no way dependent on the rhythm of the stimulus.

The appearance of all these synaptic reactions in the retina is particularly interesting because they serve no very obvious purpose. No doubt it would be possible to find a teleological explanation, for instance the spread of excitation allows a dim light to have a considerable stimulating effect if it illuminates a large area of the retina, and this may be of advantage to a marine animal: but it seems more likely that the spreading is a necessary consequence of the structure of the retina, i.e. of the fundamental properties of the network of dendrites and synapses which intervenes between the nerve cell layers. The rhythmic discharge from the eel's eye must be another manifestation of the fundamental properties of a group of associated neurones. It can be of very little importance in the normal working of the eye, for it does not appear unless the whole retina is evenly illuminated and then only when the excitation has fallen considerably from its initial intensity.

Conduction in the eel's retina is therefore a process exhibiting many of the phenomena which may occur whenever a large group of neurones can establish synaptic linkages with one another, and in support of this

## CONCLUSIONS.

As far as concerns the physiology of vision the main bearing of the present experiments is to emphasise the fact that the eel's retina is not a mosaic of receptors or groups of receptors with each group leading by an independent pathway to the corresponding optic nerve fibre. No doubt there is a closer connection to one particular fibre, but the same fibre is open to the spread of excitation from other parts of the synaptic layers. In our own eyes we must suppose that the nervous pathways (for the cones at least) are more restricted, but the existence of a considerable amount of interaction is shown by the way in which the size of the retinal image influences the threshold for colour and the fusion point for flicker as well as the threshold for colourless light. These facts by themselves are enough to throw doubt on any theories of vision in which the cones and their connections with the optic nerve fibres are treated as independent units.

Apart from this, however, our results show that the process of conduction through the retina has many of the features of conduction in a reflex arc, as we might expect from its structure. There is a variable latency depending on a gradual summation of excitations and the possibility of a widespread irradiation of the excited state in the synaptic layers. Another feature of the discharge which might be cited is the occurrence of the "off effect"—the renewed outburst of impulses on the extinction of a light which has acted for more than a second. The likeness of this to the terminal rebound in a reflex contraction is close enough to suggest a common origin. Whether the decline in the discharge with steady illumination is comparable with the fatigue of a reflex arc is more doubtful; the same decline in frequency occurs with the peripheral sense organs, though we need not assume that the "adaptation" of a sense organ and the "fatigue" of a reflex arc are radically different.

The rhythmical strychnine discharge in the optic nerve has already been likened to that from the spinal cord, and there are several instances of the synchronous action of large groups of neurones without strychnine. Thus Gasser and Newcomer<sup>(1)</sup> have shown that the motor neurones innervating the diaphragm must work more or less in unison, and the occurrence of a dominant rhythm in the electromyogram of a voluntary contraction is best explained in the same way. It is true that in both examples a rhythmic stimulus acting on a number of independent neurones might account for their synchronous activity. In fact the

rhythm might be comparable to that produced in the optic nerve by a flickering and not a steady light. Explanations on these lines have often been advanced to account for the rhythm in the electromyogram (12). There is one case, however, where the synchronisation must be due to interaction in the cord itself; this is the experiment made by Dusser de Barenne and Brevée (13) in which the dorsal part of the cord was treated with novocaine, and the electromyogram of the contraction due to touching the cord became more and more regular as the sensory neurones were put out of action. The electromyogram has been mentioned because in one respect it shows a remarkable likeness to the optic nerve discharge. With a feeble contraction there is no sign of the dominant 50 a sec. rhythm; to produce it the contraction must be powerful. *i.e.* a large number of motor neurones must be involved. To produce the rhythmic discharge in the optic nerve the whole retina must be illuminated and here too there is no sign of a dominant rhythm when only a few neurones are activated.

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Conduction in the eel's retina is therefore a process exhibiting many of the phenomena which may occur whenever a large group of neurones can establish synaptic linkages with one another, and in support of this



we may turn to what is perhaps the most interesting point in connection with the rhythmic discharge, namely its tendency to appear only after repeated exposures to a uniform light. Some preparations (particularly those used in the summer months) gave the rhythmic discharge during the first period of illumination, but in every case the rhythm appeared with much greater certainty towards the end of an experiment when repeated exposures had been made. It does not seem likely that this can be due to fatigue, for the eye will be more fatigued at the end of a long first exposure of several minutes than in the early stages of a subsequent exposure made after a rest of 5 or 10 minutes in the dark. Nor is the rhythm favoured by mere rest in the dark or by repeated stimulation of a restricted area. Indeed the rhythmic tendency is sometimes impaired if the retina is illuminated over a restricted area in the course of the experiment and it is then necessary to build it up again by several periods of uniform exposure.

Since the ability to produce a synchronous discharge persists throughout the periods of darkness between successive exposures there must be some more or less permanent increase in the power of interaction between the retinal neurones, *i.e.* more widespread connections must be developed, and the factor which promotes them seems to be the repeated activity of the neurones. The lasting facilitation which occurs when a point on the surface of the cortex is stimulated and the direction of impulses from other points into the same channels ("deviation of the response"—Sherrington) shows that the activity of a group of neurones may cause temporary connections to be established over a wide area and the whole working of the cortex seems to depend on reactions of this type. That the same process can occur in a minor degree in the simpler nervous apparatus of the retina is a possibility which is clearly too interesting to be dismissed, though its discussion must be postponed until we have more certain evidence.

#### SUMMARY.

1. When the entire retina of the Conger eel is exposed to uniform illumination the action current discharge in the optic nerve may lose its usual irregular character and may consist of a series of regular waves with a frequency which usually varies between 15 and 5 a sec.

2. These waves are comparable with those observed by Fröhlich in the electric response of the cephalopod eye, though the latter have a higher range of frequency. In both cases the frequency declines as the exposure is continued and varies with the intensity of the light.

3. The waves in the optic nerve discharge of the eel are caused by a rhythmical waxing and waning in the number of impulses in the nerve fibres. It follows that the ganglion cells of the retina must all be working in unison with alternating periods of rest and activity.

4. A discharge of this kind might be caused by a rhythmical stimulation of the retina such as that occurring with a flickering light, and it is true that records of the response to flicker are rhythmic over much the same range, i.e. the response becomes irregular when the rate of flicker exceeds about 15 a sec. The exact fusion point depends on the intensity of the light and the size of the area illuminated.

5. An alternative explanation is that the ganglion cells discharge in unison because of nervous connections between them. If so the rhythmic response should be favoured by strychnine, for strychnine can promote synchronous discharges from large groups of neurones in the spinal cord.

6. In preparations which do not normally show any rhythm, strychnine has an immediate effect. It produces rhythmical discharges from 2-8 a sec. in the dark and, later, rhythmic discharges from 15-5 a sec. during exposure to light.

7. Since the effect of strychnine on the retina corresponds so closely to that on the spinal cord, we conclude that the rhythmic discharges from the retina are due to nervous interconnection between the ganglion cells and not to intermittent stimulation of the rods and cones.

8. The synaptic layers connecting the different bipolar and ganglion cells seem unable to beat synchronously with a rhythm higher than about 15 a sec. This would account for the fact that the discharge does not become rhythmic until the light has been in action for 5-10 sec.

9. When four points on the retina are illuminated simultaneously, the reaction time of the optic nerve discharge is shorter than when any one of the points is illuminated alone. Strychnine causes a marked increase in the range over which this interaction can occur, and we conclude that it is due to a nervous summation in the synaptic layers of the retina.

10. The parallel effects of an increase in the intensity of the light and an increase in the size of the illuminated area must be due in the same way to a nervous summation of the excitation from different points.

11. The nature of the nervous connections in the retina is discussed. These are found to have many of the properties shown by the grey matter of the central nervous system. In the eel's eye there can be no exclusive nervous connections between one group of retinal elements

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# STUDIES IN THE PULMONARY CIRCULATION.

## I. The vaso-motor supply.

BY W. E. DIXON AND J. C. HOYLE.

*(From the Pharmacological Laboratory, Cambridge.)*

It is now twenty-three years since T. G. Brodie and one of us<sup>(1)</sup> gave reasons for believing that the pulmonary blood vessels were not innervated. Previously it was held that these vessels received an efficient vaso-motor supply from fibres leaving the spinal cord in the anterior roots of the 2nd to the 7th dorsal nerves. The new doctrine suggested that the blood supply to the lungs was regulated by the right heart and that an independent vaso-motor system was unnecessary. This view led to the publication of some fifty to sixty papers dealing with the subject directly or indirectly, of which about half supported the findings of Brodie and Dixon. The only paper which produced evidence showing any considerable degree of pulmonary vaso-constriction was that of Fühner and Starling<sup>(2)</sup>. These observers, using the heart-lung preparation, obtained a rise in pulmonary arterial pressure with adrenaline accompanied by a fall in left auricular pressure and a constant or slightly decreased heart output. This effect we know is due to increased coronary outflow<sup>(3)</sup>.

More recently the subject has been reviewed very thoroughly by Schafer and Lim<sup>(4)</sup> and Wiggers<sup>(5)</sup>. After reading through the literature we are left with the impression that occasionally under good physiological conditions perfusion experiments may show slight constriction of vessels either after the injection of adrenaline or stimulation of the upper thoracic sympathetic system. These effects, however, are always trivial and do not appear to us to be likely to have any serious practical significance in the regulation of the pulmonary circulation. They have been made on surviving organs perfused either with blood or blood and Ringer, and sometimes with Ringer alone.

To make the position conform with the many observations which have been made to determine the distribution of vaso-motor nerves to other organs we determined to excite the spinal and sympathetic nerves, as did Bradford and Dean<sup>(6)</sup>, whilst recording the alterations in the

and one ganglion cell, and the evidence of interaction in the human eye points to the same conclusion.

The expenses of this research were largely defrayed by a grant to one of us (E. D. A.) by the Government Grants Committee of the Royal Society.

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vascular volume of the lungs. Bradford and Dean recorded pulmonary pressure and found an increase in the pulmonary arterial pressure on exciting the sympathetic nerves which they interpreted as due to constriction of vessels. This effect is now usually explained, as it was by Brödie and Dixon, by the simultaneous stimulation of the augmentor and accelerator fibres to the heart. To avoid this interference our observations were made in a number of animals after severing the cardiac fibres, leaving only the pulmonary fibres intact.

### *Method.*

Cats and dogs were used throughout for the experiments; they were anaesthetised, dogs usually with morphine and ether and cats with ether and urethane. In the early experiments curare was given in sufficient amount to paralyse the motor nerves. The thoracic viscera were then exposed from the right side by the removal of the upper four or five ribs from their sternal ends as far back as possible, the intercostal arteries being ligatured. The ganglion stellatum and the adjacent sympathetic fibres were now exposed and the fibres traced a short distance in their passage to the heart and lungs. When the dissection rendered the branching fibres sufficiently obvious each nerve was excited electrically and those which augmented or accelerated the heart-beat, as determined by inspection and the effect on blood-pressure, were severed. Finally the exclusion of augmentor and accelerator fibres to the heart was tested by stimulating all the fibres passing through the ganglion stellatum whilst observing the heart-rate and systemic blood-pressure, the experiment being regarded as unsatisfactory if any increase occurred in either.

The lung-volume was recorded in the usual way by the Brodie-Dixon method, with an air tambour connected to an oncometer enclosing the middle lobe of the right lung. The bronchial airway to that lobe was plugged in nearly full inflation with vaselined cotton-wool. The systemic blood-pressure was recorded usually from the right carotid artery. Artificial respiration was made with a Brodie's pump, using warm moist air. An ordinary laboratory coil with platinum electrodes was used for stimulation: the current could just be felt on the tongue with the secondary coil at 16 cm. and was uncomfortable with the coil at 9 cm.

### *Experimental.*

If the sympathetic nerves passing through the stellate ganglion are stimulated electrically with the faradic current in the dog or cat, the

heart-beat is accelerated and augmented, the pulmonary and systemic blood-pressures rise, and the vascular volume of the lung increases. This effect is constant, though the extent of the rise in pulmonary pressure varies in different animals and also the amount of acceleration of the heart. This effect on lung-volume is illustrated in Fig. 1 in the

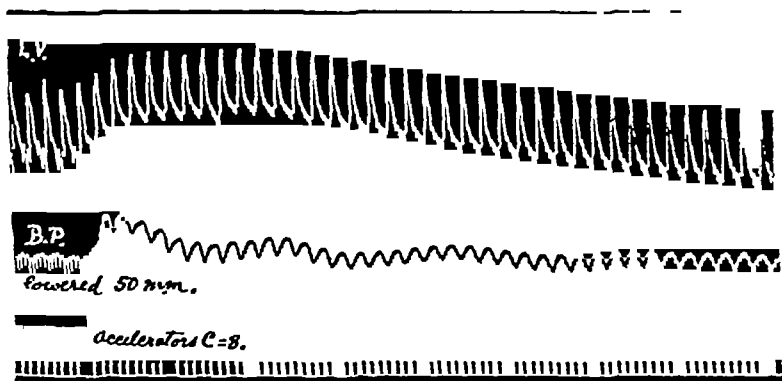


Fig 1. Dog. Morphine, curare, ether. Bronchus plugged. Lung-volume and blood-pressure. Shows effect of stimulating main accelerator nerves with coil at 8 cm.

case of the dog. The effect is not due to contraction of the pulmonary vessels. This is easily shown by placing a cannula in one of the smaller pulmonary veins and counting the drops per minute: on exciting the sympathetic nerves the outflow increases usually by about 30 p.c. During the period of stimulation the left auricular pressure also rises from 6–10 mm. saline. That is to say during stimulation of the sympathetic the whole pulmonary system becomes congested.

Bradford and Dean based their evidence for the existence of vaso-constrictor nerves to the pulmonary vessels largely on pulmonary pressure records. In certain of their experiments no acceleration of the heart-beat was observed on stimulating sympathetic fibres; they lay stress on this as affording evidence that cardiac action was lacking. They produce no evidence however to show that augmentor action was also lacking. Like Bradford and Dean we have occasionally found that stimulation of these sympathetic fibres causes no appreciable quickening of the heart, but augmentation is the rule and the effect of this is so profound on the pulmonary circulation as to invalidate any conclusions which may be drawn as to the condition of the pulmonary vessels.

When the stimulation of the sympathetic nerves causes no quickening



of the heart-beat but only some augmentation we have on three or four occasions seen some trifling suggestion of pulmonary vaso-constriction on the lung-volume record. Fig. 2 shows such an effect in the cat. In all these experiments the apparatus was arranged to exhibit a very large volume-pulse thus affording every opportunity for registering slight changes. It is obvious from Fig. 2 that the degree of constriction with

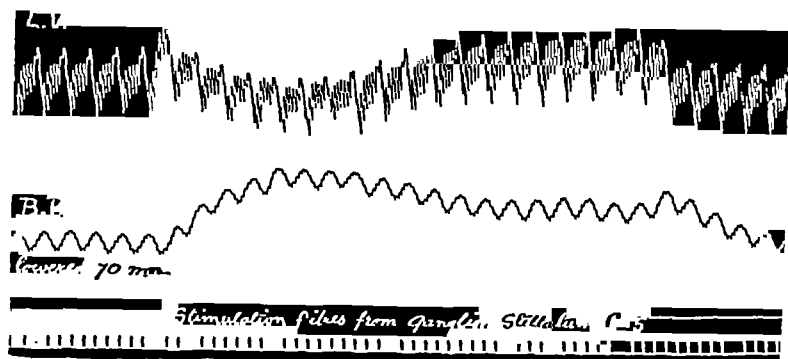


Fig. 2. Cat. Curare, ether. Bronchus plugged. Lung-volume and blood-pressure. Stimulation of the chief group of fibres from the ganglion stellatum, coil at 5 cm.

a strong stimulation extending over four minutes is very slight. At first there is a slight diminution in the volume-pulse and lung-volume but this soon gives place to an increase in both, even while the stimulus lasts. Acceleration of the heart-beat is negligible but augmentation is obvious from the rise in blood-pressure.

The cardiac fibres in the sympathetic seem to be more sensitive to exposure and temperature changes than the pulmonary fibres, since on two occasions after an unduly prolonged preliminary operation, stimulation of the accelerators has produced no decided action on the heart, but, after a transient dilatation, a definite fall in the lung-volume. Fig. 3 shows such an effect: in this figure it will be seen that the blood-pressure remains unchanged throughout. Furthermore during a prolonged experiment the cardiac effects of sympathetic stimulation, pronounced at first, become less after each stimulation and may disappear entirely. These curves (Figs. 2 and 3) are interesting but of little real significance; they were obtained in only three out of twelve animals and the degree of constriction is quite trifling. Perhaps the crucial test of vaso-constriction in these cases is change in volume-pulse, and this remains practically unaltered.

The crucial experiment to determine whether or not vaso-constrictor nerves pass to the lungs must be made by stimulating these nerves only.

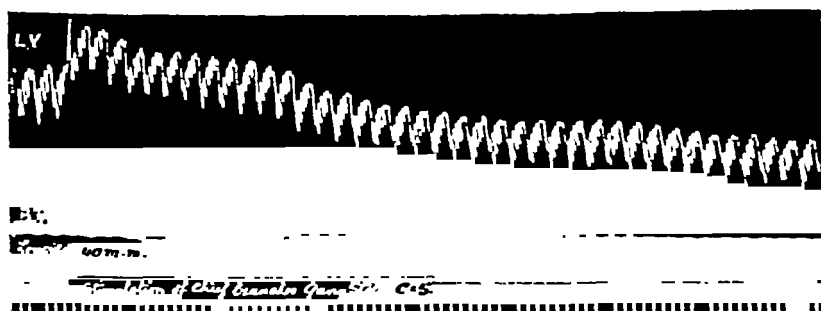


Fig. 3. Cat. Urethane. Bronchus plugged and vagi cut. Lung-volume and blood-pressure. Stimulation of the chief branches of the ganglion stellatum, coil at 5 cm.

To do this we adopted the tedious method of severing all the nerves going to the heart, adopting the precautions previously mentioned for ensuring that this operation was complete. This we have performed successfully four times, and in each of these experiments stimulation of the fibres passing through the ganglion stellatum led to some diminution

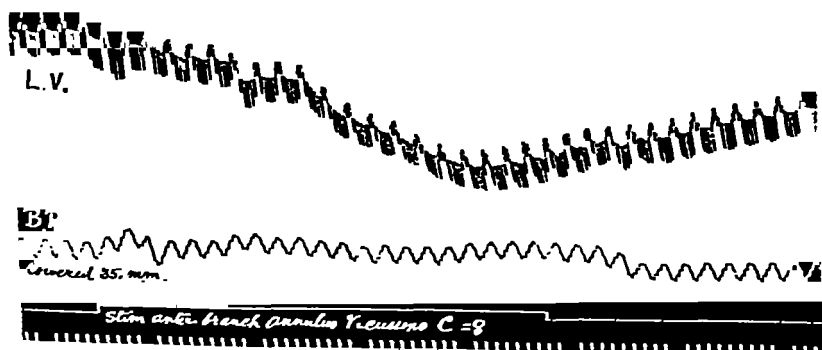


Fig. 4. Cat. Morphine, ether. Bronchus plugged and cardiac nerves divided. Lung-volume and blood-pressure. Stimulation of the anterior branches of the annulus of Vieussens, coil at 8 cm.

in the lung-volume. Fig. 4 is a typical observation. Both the lung-volume and volume-pulse diminish during stimulation and both return

of the heart-beat but only some augmentation we have on three or four occasions seen some trifling suggestion of pulmonary vaso-constriction on the lung-volume record. Fig. 2 shows such an effect in the cat. In all these experiments the apparatus was arranged to exhibit a very large volume-pulse thus affording every opportunity for registering slight changes. It is obvious from Fig. 2 that the degree of constriction with

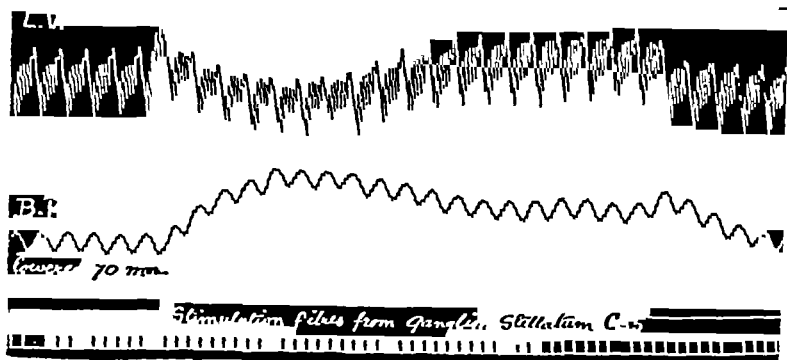


Fig. 2. Cat. Curare, ether. Bronchus plugged. Lung-volume and blood-pressure. Stimulation of the chief group of fibres from the ganglion stellatum, coil at 5 cm.

a strong stimulation extending over four minutes is very slight. At first there is a slight diminution in the volume-pulse and lung-volume but this soon gives place to an increase in both, even while the stimulus lasts. Acceleration of the heart-beat is negligible but augmentation is obvious from the rise in blood-pressure.

The cardiac fibres in the sympathetic seem to be more sensitive to exposure and temperature changes than the pulmonary fibres, since on two occasions after an unduly prolonged preliminary operation, stimulation of the accelerators has produced no decided action on the heart, but, after a transient dilatation, a definite fall in the lung-volume. Fig. 3 shows such an effect: in this figure it will be seen that the blood-pressure remains unchanged throughout. Furthermore during a prolonged experiment the cardiac effects of sympathetic stimulation, pronounced at first, become less after each stimulation and may disappear entirely. These curves (Figs. 2 and 3) are interesting but of little real significance; they were obtained in only three out of twelve animals and the degree of constriction is quite trifling. Perhaps the crucial test of vaso-constriction in these cases is change in volume-pulse, and this remains practically unaltered.

The crucial experiment to determine whether or not vaso-constrictor nerves pass to the lungs must be made by stimulating these nerves only.

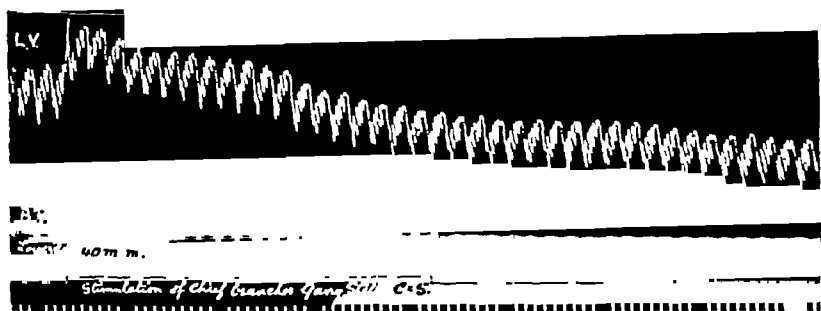


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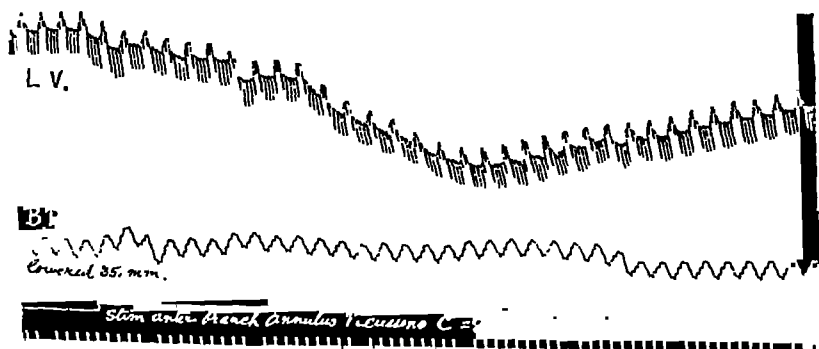


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in the lung-volume. Fig. 4 is a typical observation. Both the lung-volume and volume-pulse diminish during stimulation and both return

to the normal when the stimulation ceases. This effect was constant in each experiment though it varied in degree, and the constriction persisted only during the period of stimulation. After exposure the effects of stimulation became less and less evident and ultimately ceased. The blood-pressure remained practically unchanged.

It may be argued that these experiments afford direct evidence of pulmonary vaso-constrictor nerves. But they also show that even under the conditions of these experiments which were devised to show the most minute changes, the degree of constriction is negligible.

In conclusion we claim that these crucial experiments devised and based upon the methods adopted for determining the presence and distribution of vaso-constrictor nerves to other organs, show both comparatively and absolutely no evidence of an effective vaso-motor supply to the pulmonary system. The degree of constriction which we have described approximates rather to a "vestigial" effect.

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## EXTENSOR REFLEXES IN THE FORE-LIMB.

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It was described in a previous communication<sup>(1)</sup> that "extension and not flexion readily occurs from stimulation of the nerves or nerve-endings of the fore-limb" and was suggested that "the result may be due to the mixed character of the nerves." Further investigation has been made on this propensity towards extension in the fore-limb of cats on stimulation of ipsilateral nerves and the results are here described. *M. supraspinatus*, an anti-gravity muscle of the shoulder, again has been used, as well as *M. biceps brachii* and *M. brachialis*, to indicate the reflex response ensuant on nerve stimulation.

### METHOD OF EXPERIMENT.

The cats were decerebrated under profound C.E. anæsthesia by the trephine method and section of the brain stem at levels corresponding to those described and illustrated as Sections I to IV in Magnus' *Körperstellung*(s). The left fore-limb was used. Usually only one or two of the muscles observed (*M. supraspinatus*, *M. biceps brachii*, *M. brachialis*) were isolated for any one experiment. All other muscles in the limb were paralysed by nerve section or resection. Nerves selected for stimulation were ligated and isolated from surrounding tissues. At one time or another during this research all accessible peripheral nerves of the fore-limb were tested for their reflex effect on the observed muscles, especially the median nerve in the arm, forearm, and wrist, the ulnar in the arm and wrist, the internal cutaneous, the circumflex, the nerve to latissimus, the subcapulars, the branches of musculo-cutaneous to biceps and to brachialis, and the musculo-spiral nerve and its branches. Twist drills were inserted into the humerus just below the head and just above the epicondyle's fixation of the upper end and of the lower end of the humerus respectively. The scapula was held by two small stout brass bone-clamps, one applied at its vertebral border just caudal to the spine of the scapula, the other to the metacromion.

These bone-clamps checked especially lateral movement. Rotational movements were prevented by inserting two drills into the scapula, one at a point on the edge of the vertebral border near the clamp, the other near the spine and just dorsal to the metacromion. The drills and bone-clamps were held by very stout universal joint-clamps to steel uprights which in their turn gripped the oaken top of a heavy experimental table. The tendon of the muscle was arranged without deviation from the natural line to pull vertically downwards on the myograph. This necessitated the preparation lying on its back on the table, though the head could be turned laterally. The myograph was an isometric shadow myograph of high vibration frequency (1000 dv. per sec.) similar to that used previously, incorporated in the same optical system as a string galvanometer (new Cambridge pattern). The tendon of the muscle observed was attached to the horizontal arm of the myograph by a light steel hook, often in conjunction with a short length (1 cm.) of stout fishing line. On occasion we used a two-wire myograph in conjunction with a double string case in the galvanometer for taking simultaneous records from muscles such as *biceps brachii* and *brachialis anticus*. Ag. AgCl electrodes led from the muscle to the galvanometer. The string tension was usually 5 mm. per 1 mv. For electrical stimulation of nerves, Sherrington torsion wire keys were used to interrupt a 2-volt primary circuit of either Berne or small class coils (without core). The opening and closing of short-circuiting keys were photographed directly on the plate. The stimulating electrodes were hand applied, their points being of silver wire.

*Median nerve. (Ipsilateral.)*

This ipsilateral nerve evokes an interesting response from a presumptively typical extensor muscle like *M. supraspinatus*. It is very frequent to find that when this nerve is stimulated at the level of the wrist joint (that is, after it has given off its branches to muscles of the forearm) it evokes a contraction in the ipsilateral *M. supraspinatus* which may develop an active tension of as much as 0.75 kg. within 1 sec.<sup>1</sup> (Fig. 1). The development of tension is gradual and recalls to mind the process of "recruitment" of motor units in the crossed extensor response of the lower limb, and may on occasion bear all the features of such recruitment, that is, a long latent period, the sigmoid rise to a plateau and a considerable after-discharge. Often, however, there is

<sup>1</sup> Direct stimulation of the motor nerve of *M. supraspinatus* of a cat weighing 2.5 kg. (a weight usual with us) produces a tension of 7.0 kg.

a departure from the typical sigmoid curve and the rise of tension, once it has begun, is more linear, while on withdrawal of the stimulus there

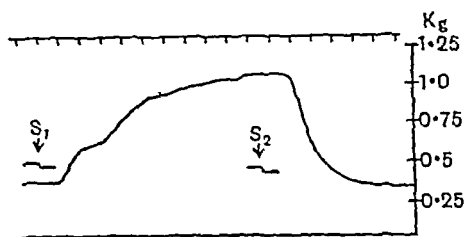


Fig. 1. Supraspinatus. Stim. of ipsilat. median nerve at wrist, coreless Berne coil 13 cm., 55 break-shocks per sec. Time 0.1 sec. at top of figure. Tension scale shows line of zero tension.  $S_1$ , onset of stimulus;  $S_2$ , cessation of stimulus. This and all figures read from left to right.

may be marked rebound. The presence of rebound after the stimulus shows that there has pre-existed a state of inhibition. Not infrequently there is an absence of after-discharge and of rebound, the termination being abrupt and angular with corresponding quietude in the string and suggestive of latent inhibition. The linear rise in tension is possibly the resultant of the two opposing processes, excitation and inhibition. The inhibition may be evident *ab initio* if a moderate tension of active reflex stretch is present, for then at the onset of the stimulus the tension falls rapidly, indicating inhibition, and subsequently after the usual long latent period (itself possibly a result of mixed excitation and inhibition) the contraction of the muscle develops in the characteristic manner. More often, however, the pre-existence of a degree of active reflex tension ("stretch-reflex") is necessary before the excitation itself appears in response to stimulation. For example, should the resting tension be slight, the stimulation of the nerve produces either no, or a very small degree of, excitation. If the resting tension be raised a little (e.g. to 0.025 kg.) the excitatory response is now appreciable. On further raising the tension (e.g. to 0.7 kg.) the amount of the excitatory response remains the same when it develops, but it is preceded by an inhibitory fall and reveals itself in the trough of inhibition (Fig. 2). At high tensions the inhibitory trough is correspondingly deeper, while the excitation is but little altered. The dependence on a moderately high degree of initial tension for its appearance seems to be an essential characteristic of the reflex, and the degree of stretch is proportionately higher than that needed for any other reflex so far investigated, e.g. the crossed extensor response, which latter though noticeably very responsive to small



changes in initial tension does not demand initial tensions as high as 0.3 kg. for its appearance (Fig. 1). This marked dependence on the

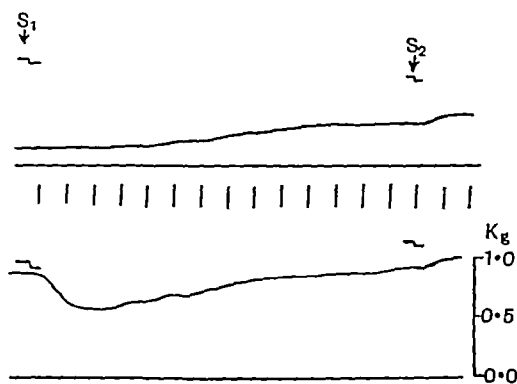


Fig. 2. Supraspinatus. Stim. of ipsilat. median nerve at wrist, coreless class coil 8 cm., 48 break-shocks per sec. Time 0.1 sec. Tension scale applies to both tracings. Upper tracing at low initial tension (0.025 kg.), lower tracing at higher initial tension (0.7 kg.).  $S_1$ , onset;  $S_2$ , cessation of stimulus.

degree of initial tension, which is itself in large part the expression of resting "posture" (tone) or stretch-reflex, suggested that modification in the response might be obtained through the neck afferents or the labyrinth. We have not obtained, however, results of any constancy up to eight hours after decerebration although the crossed extensor response, even after adjustment of the initial tension, has shown evidence of the effect in the one and the same preparation by an abruptness of onset and a high plateau of tension in the "maximum" position of the head and neck. Further observations at a longer interval from decerebration are desirable. A characteristic of this reflex is its susceptibility to the concurrent stimulation of other ipsilateral nerves. The upper branches of the median nerve by themselves constantly exert a profound inhibition on it, evoked as it is from the lower branch of the median nerve to the hand (Fig. 3 a, b). In the preparation from which these tracings were taken the forearm branch of median produced no evident effect on the muscle in either direction. Yet when this branch is stimulated during the excitation evoked by the wrist branch there is a profound inhibitory fall, almost to the initial level of tension. The tracing, in comparison with the uninhibited response, is typical of the process of inhibition, although, in so far as inhibition has been most completely studied in the hind-limb muscles, it must be remarked that here there is a long latent period and a not very rapid decline as has been previously noted (5).

This low tension is attained if the whole median trunk in its upper course (including both the branches) be stimulated maximally during a stretch-

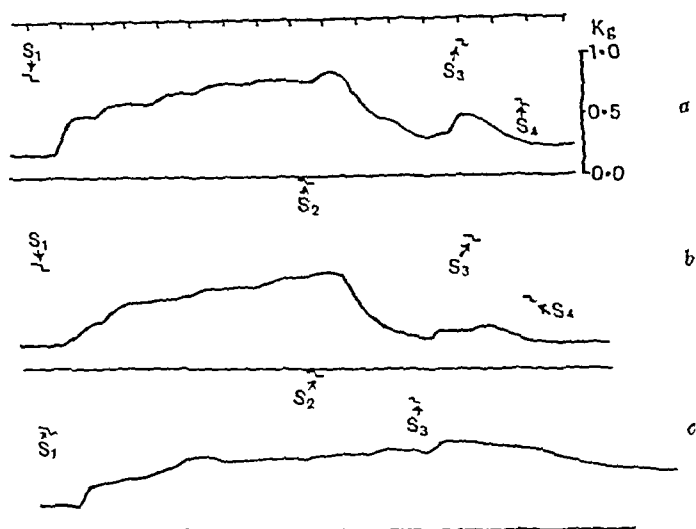


Fig. 3 a. Supraspinatus. Stim. of ipsilat. median nerve at wrist, coreless class coil 7 cm., 48 break-shocks per sec., between  $S_1$  and  $S_3$ , with stim. of forearm br. of ipsilat. median, Berne coil coreless, 10 cm., 50 break-shocks per sec., between  $S_2$  and  $S_4$ . Time 0.1 sec.

Fig. 3 b. Similar, but stim. of forearm br. now stronger, Berne 9 cm.

Fig. 3 c. Control of stim. of median nerve at wrist only.

reflex, and it is evident therefore that the branch to the fore-limb muscles is mainly responsible for the non-appearance of excitation in this case. With a slightly weaker stimulus to the upper trunk of median there is before the decline a slight rise, indicative of excitation, which even in the hind-limb is not uncommon (Fig. 3 a), while correspondingly after the first fall there is a marked increase in tension suggestive of the slow development of an excitatory process. On occasion it has been possible to obtain with a weak stimulus a rise of excitation from the whole median equivalent to or even greater than that produced by its wrist division. This excitation could be totally inhibited by adding an intercurrent stimulus to the forearm branch of the same nerve. It is evident then that the excitation of the whole trunk with a weak stimulus did not involve the fore-limb branch, and we venture to suggest that the varying degrees of excitation and inhibition resulting from stimulation of the whole median trunk are the result of conflict between the two factors found almost pure in its two lower divisions.

In confirmation of the inhibitory action of the forearm branch of the median nerve, we have found that stretch applied to the tendon of *M. flexor digitorum profundus* (the ulnar nerve being cut in the arm) produces strong reflex inhibition on all activities of *M. supraspinatus* (stretch-reflex, crossed extensor-reflex and "ipsilaterals" from the median at the wrist). Thus a stretch of 0.5 kg. applied to the tendon of *M. flexor digitorum profundus* is adequate to produce profound inhibition in a stretch-reflex of *M. supraspinatus*. When a nerve of the other fore-limb is stimulated (the ipsilateral ulnar nerve being cut in the arm, as before), *M. flexor digitorum profundus* is excited, as well as *M. supraspinatus*. During those conditions, a stretch of 1.5 kg. to the flexor produces relaxation in it directly, and partial inhibition of *M. supraspinatus* reflexly. Similarly a stretch of 2 kg. to the flexor inhibits reflexly a tension of 2 kg. in *M. supraspinatus* almost to zero. Stretches to the flexor's tendon also excite extension in the other fore-limb.

Since these large "ipsilateral" contractions ensued in *M. supraspinatus* on stimulation of the median nerve at the wrist and after the loss of the forearm branch, we proceeded to investigate the part played by the nerve at the ultimate distribution in the digits as digital nerves. The stimulation of the digital nerves has been carried out by passing a pair of fine gilt pins subcutaneously into the side of the digit along the course of the nerve. Such a method avoids exposure and drying of these small nerves but may be held to be not above criticism on grounds of trauma and "escape of current," which might seem more probable because strong stimuli are needed to overcome electrical resistance in the cutis. We have not found in control observations that either of these factors is at all considerable. If then any one digital nerve which is a branch of the median be stimulated in this manner, the ipsilateral contraction of *M. supraspinatus* is elicited and may add to the initial tension a notably high tension (e.g. 0.45 kg.), considering the small number of nerve fibres which are excited, that is, the digital nerve has a wide distribution centrally to the spinal motor units. Further investigation of the skin field shows that the digital clefts and palmar aspect of the palm, as supplied precisely by the median nerve, are a distinct area, since the ulnar nerve as will be described later in this paper does not so behave. The external aspect of the forearm when stimulated will also excite the *supraspinatus*. This ipsilateral excitation of the median field at the digital clefts confirms then the excitation from stimulating the whole nerve trunk at the wrist, although, owing to overlap of the cutaneous fields, some stimulation of the ulnar distribution

presumably occurs, especially near the border of overlap. This is evidenced by a wavering response when a skin zone near the border line is stimulated. We have found also that a nociceptive mechanical stimulus (pinch) produces a brief response in *M. supraspinatus* when applied to the fore-paw, especially on the radial side. The nature of the response varies according to the position of the neck. Thus when the chin is turned towards the side stimulated, a pinch is followed by slight relaxation (inhibition), and when the occiput is turned to the side, slight excitation ensues.

As regards the level of decerebration in relation to these effects of *M. supraspinatus* from stimulation of the median nerve at the wrist or its digital branches, we have not found that for its development there is any dependence at all on the level, whether that be intercollicular, just precollicular, or thalamic. In the case of a section in front of the intercollicular plane massive rebound of high tension may occur after the stimulus, evidence of the latent inhibition that occurs on stimulation, while in an intercollicular preparation which may have a very high initial tension of active "stretch," inhibition may occur at the outset before the ipsilateral is built up. Section of the spinal cord in the thoraco-lumbar region is also without effect.

As the median nerve has this peculiar action in exciting a supposed extensor of the ipsilateral side, we explored its effect also on a flexor muscle of the elbow, *M. brachialis anticus*. There appears to be nothing atypical in the type of tension curve of *M. brachialis* in response to most nerves of the ipsilateral limb, for it corresponds with familiar paradigm of reflex flexor response, that is, a brief latent period, an abrupt onset, a rapid climb to a level plateau and a relative absence of after-discharge. But the ipsilateral median nerve, by its wrist division, very seldom elicits any large excitatory response with abrupt onset in this flexor in decerebrate preparations, but rather responses with a gradually climbing onset and a low tension plateau. When stimulated intercurrently in the plateau excited by another nerve (*e.g.* ulnar or musculo-spiral) or during the after-discharge from such a plateau, there is a very marked inhibitory effect. This then appears to be the reciprocal component of the excitation observed with *M. supraspinatus*, though we have not found for *M. brachialis anticus* that there is antagonism between the forearm and the wrist branches of the nerve.

*Combination of the median nerve with other nerves.*

Because the median nerve at the periphery of the limb acted in this manner on *M. supraspinatus*, it was of interest to observe the effect of other nerves, singly or in combination. A nerve closely related in anatomical distribution to the median nerve is the ulnar. In general, the ulnar nerve stimulated anywhere in the fore-limb exerts an action more markedly inhibitory than does the median at the wrist. That is to say, that although inhibition is the usual consequence of stimulating strongly the ulnar nerve in the arm or at the wrist, especially when the spinal-motor units are already excited from some source (stretch-reflex, contraction from stimulation of ipsilateral median at wrist or crossed extensor response), the nerve stimulated alone with a low initial tension may in some few preparations produce a small contraction in *M. supraspinatus*, of which the general features though less in degree resemble those excited by the median nerve, in there being a long latent period, a slow linear climb to a plateau tension and long after-discharge. A terminal rebound usually follows the contraction. On the other hand, the more usual plainly inhibitory action can be brought to bear against the median "ipsilateral contraction" with striking results, in that the contraction can be annulled to the base line by quite weak stimulation of the ulnar nerve in the arm or forearm. Its powerful antagonism to the median is even more striking when the digital nerves derived from the ulnar nerve are stimulated by the method previously described for the median digital divisions. Then stimulation of one digital branch of the ulnar nerve has a striking inhibitory action on the spinal motor units of *M. supraspinatus*, showing that the central connections of the ulnar nerve have an intimate relationship over a large part of that group of final motor neurones. Thus on occasion we have observed that a contraction in *M. supraspinatus* of some 2.75 kg. elicited by stimulation of the median at the level of the wrist can be inhibited to 2 kg. by stimulating the nerve in the border of the last digit of one and the same fore-paw (Fig. 4). An ipsilateral contraction of 0.53 kg. elicited from the internal cutaneous could be completely inhibited by the same stimulus. Further exploration of the ulnar border of the fore-limb has shown that it has an inhibitory action on *M. supraspinatus* more proximally in the area supplied by the internal cutaneous nerve. Stimulation of this nerve trunk (which has been with us the more usual method) or its skin area has centrally a strong influence on the motor units of *M. supraspinatus*. The nerve trunk stimulated by itself against a moderate initial tension

of active stretch in *M. supraspinatus* (stretch-reflex) produces a typical inhibitory fall of tension, although certain rare preparations, active

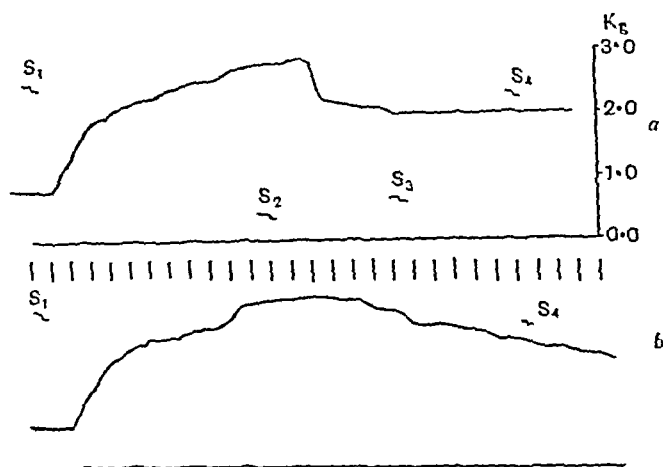


Fig. 4 a. *Supraspinatus*. Stim. of median nerve at wrist, coreless class coil, 10 cm., 43 break shocks per sec., between  $S_1$  and  $S_4$ , with intercurrent stim. of 4th digit from gilt pin electrodes in cutis, Berne 0 cm., 50 break-shocks per sec. between  $S_2$  and  $S_3$ . Time 0.1 sec.

Fig. 4 b. Control of median nerve only.

contractions (of 0.7 kg.) of the recruiting type, may develop (*e.g.* 0.8 sec.) after the preliminary inhibitory drop. With a low initial tension in the muscle the nerve seldom elicits more than a very small ipsilateral contraction of some 50 gm. tension, and on withdrawal of the stimulus. a marked rebound. The responses, in fact, are closely similar to the small ipsilateral contractions which have been described for hind-limb muscles, where nerves are more purely inhibitory. When the skin area of the internal cutaneous nerve, to give one example (Fig. 5 a), is stimulated intercurrently while the motor units of *supraspinatus* are being reflexly excited by stretch of the tendon (stretch-reflex), there is a sudden (within  $100\sigma$ ) and abrupt fall of tension to a low level (complete in  $300\sigma$ ), only a little above the initial tension and corresponding to the small excitation produced by the internal cutaneous alone. In contrast to this, and in an observation immediately following, stimulation of the skin of the outer (radial) border of the forearm was found to produce some contraction in *M. supraspinatus*, though slight and unsteady (Fig. 5 b), and there is evidently an inhibitory action also at play at the motor units, as the weak contraction is followed by a rebound. The

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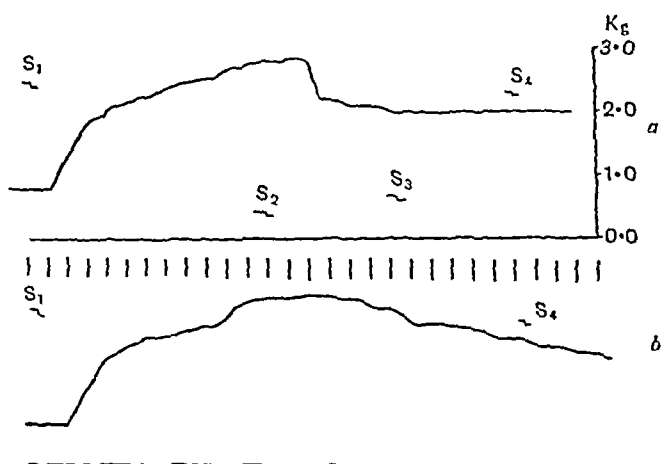


Fig. 4a. Supraspinatus. Stim. of median nerve at wrist, coreless class coil, 10 cm., 48 break-shocks per sec., between  $S_1$  and  $S_4$ , with intercurrent stim. of 4th digit from gilt pin electrodes in cutis, Berne 0 cm., 50 break-shocks per sec. between  $S_2$  and  $S_3$ . Time 0.1 sec.

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complete musculo-spiral nerve containing the branch to this area stimulated in the arm if stimulated maximally is the most powerful

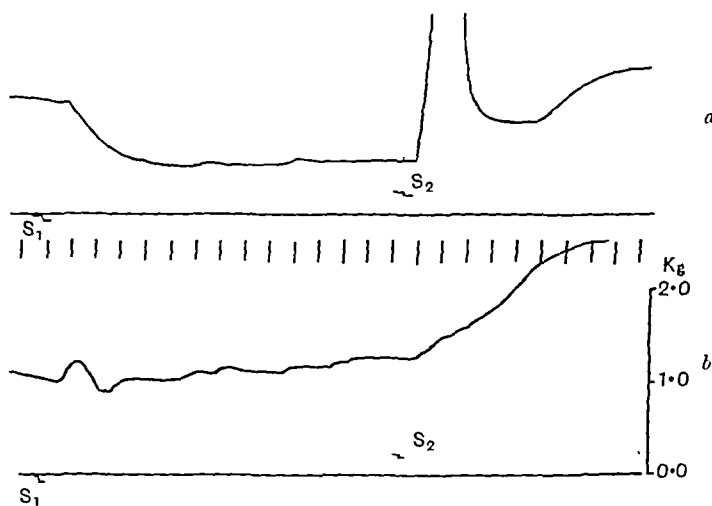


Fig. 5 a. Supraspinatus. Tetanic stim. (during stretch-reflex) from gilt pin electrodes in cutis of inner border of forearm, between  $S_1$  and  $S_2$ . Time 0.1 sec.

Fig. 5 b. Same, of outer border of forearm.

inhibitor of *M. supraspinatus* that we have found. Thus the musculo-spiral nerve, inhibitory as a whole, and yet excitatory in its relatively small cutaneous division, has the same two contrasted properties as have the trunk and wrist division of the median nerve, already described. The inhibitory action of these nerves on the extensor phase of the fore-limb has its complement in the excitation which is promoted simultaneously in a typical flexor muscle such as *M. brachialis anticus*. The musculo-spiral nerve evokes by far the largest reflex response in *M. brachialis*, a fact indicative of a wide representation among the spinal motor units of this muscle (flexor) and confirmatory of its powerful influence in the inhibitory sense on the units of *M. supraspinatus* (extensor).

On grounds of similarity with the flexor muscles of the hind-limb it might be expected that the effect of a nerve such as the ulnar on the contralateral side would be similar to that of a contralateral nerve in the hind-limb. It has been found that the contralateral ulnar nerve maximally stimulated during the contraction of *M. brachialis* from the stimulation of the ipsilateral ulnar causes an onset of inhibition within  $60\sigma$ ,

but the effect is not profound as judged by the curve of muscle tension which declines, to quote one example, from 1.45 kg. to 0.9 kg. in 150 $\sigma$ .

It is of interest in this connection to discuss the string galvanometer record from a flexor during crossed and ipsilateral inhibition. In the ipsilateral activation of brachialis the action currents are regular and follow the rate of the exciting stimulus as in hind-limb flexors. We have not yet seen complete inhibition of a fore-limb flexor by either crossed or ipsilateral nerve, but in cases where the remainder is very low, for example, when the ipsilateral median inhibits the after-discharge of a contraction produced by ipsilateral ulnar so as to bring the tension from 1.3 kg. to 0.5 kg., the string during the inhibitory period shows only the small rhythm which is produced by the inhibitory stimulus when applied alone. With background stimuli which can be inhibited in less degree the action currents are a combination of both exciting and inhibiting stimuli. If the latter two are arranged so as to beat at a slow rate, for example when the exciting stimulus is at the rate of 50 a second and the inhibitory stimulus 51 a second, then the record during the inhibitory trough will show "noding" corresponding to the difference in rate, in the above instance at the rate of one a second. This noding occurs only when there is myographic evidence of a combined excitation and is therefore explicable on the assumption of interference of the activating rates at excited units only. As shown by Cooper and Denny-Brown it may occur when no inhibition is present(4). How much of this noding is due to occlusion and how much is not, can only be decided by the mechanical record, and we have considerable evidence to show that in many of the residual excitations there is a great degree of overlap (cf. (3)). As noding is a phenomenon which can be observed when mechanical inhibition is absent, it is not surprising to find that when inhibition is present the mechanical troughs of inhibition do not bear any temporal relationship to the noding of the string record. It occasionally happens that when internal cutaneous or median stimulated alone present a low recruiting flexor contraction they can cause a rhythmic discharge if played against a contraction background produced by another nerve. From the progression movements which occur in the hind-limbs at the same moment it is assumed that this too is progression in the fore-limb. On two occasions this rhythmic movement has coincided in rate with the difference in rate (2.5 a second) of the two sets of stimuli concerned. The rhythmic accessions of contraction were added to the background and no inhibition of the background occurred. In these cases the action currents of each accession were an amplification of the background rate.

In another case inhibition of the background formed the trough of each rhythmic step, but the rate of stepping (3.5 a second) here bore no relation to the rate of stimulus interference (2 a second).

Crossed inhibition of brachialis is never complete as it has always carried with it a small recruiting excitatory component with small irregular action currents resembling those of a small crossed extension, in which asynchronism of spinal volleys is well recognised. This crossed excitation of flexors is enhanced by an ipsilateral stimulus, so that although the crossed ulnar, for example, partially inhibits the ipsilateral ulnar the action currents during the inhibition become smaller and grossly asynchronous, and show no nodding with interfering stimuli, while the crossed ulnar alone can produce only one or two widely separated volleys. This effect is the expected result of the combination of a regular and irregular rhythm.

Judged myographically an ipsilateral nerve may have considerable excitatory overlap with its neighbours in the motor units of a flexor and only a small degree of inhibitory overlap. This is the general rule. Thus, in one experiment, the ipsilateral ulnar nerve excited a tension of 1.85 kg. in *M. brachialis anticus*. The ipsilateral median nerve, within 20σ of its stimulation, brought a fall in tension in such a plateau to 1.45 kg., while by itself it produced a tension of 0.9 kg. This latter tension, however, was not the true index of median's excitatory power, since a terminal rebound in its record showed that slight inhibition had been occurring. It was interesting to note, in that experiment, that the nerve to such a closely related muscle as *biceps brachii*, excited intercurrently in ulnar's plateau, produced a fall closely similar to that produced by median. and of the same degree. The same effect was found when the biceps muscle itself was subjected to a stretch during the contraction of brachialis anticus, care being taken to exclude passive movement as far as possible (which at the end of the experiment was found in fact to add slightly to the tension of *M. brachialis*), for then a stretch of biceps of 1.0 kg. produces a fall in tension in brachialis by one-third. This has been repeatedly confirmed and is a surprising result on the supposition that *M. biceps brachii* is a collaborating flexor of the elbow.

An ipsilateral nerve which as a general rule is an inhibitor of *M. brachialis anticus* in the internal cutaneous nerve. Thus on occasion pitted intercurrently against a tension of 0.5 kg. produced from the ipsilateral musculo-spiral nerve (class coil 13 cm.), it brought about a fall in tension to only 0.05 kg. and within 150σ of its application and 40σ of its beginning to take effect. It is noteworthy that this nerve, to the

action of which we have already adverted as an inhibitor of the shoulder's extensor, should have the similar inhibitory action on the elbow's flexor. Even when a single break-shock is applied to the nerve, it may bring about a fall of 0.15 kg. (one-seventh of the whole) within  $42\sigma$ . It seems to be peculiar, too, in its proneness at quite weak strengths of stimulation to promote running movements in the hind-limbs, and occasionally in the fore-limb. But even this nerve's effect may, in some uncommon preparations, be one of addition and not subtraction from a pre-existing plateau of tension. The additive effect, however, is small, and at most 0.1 kg. in our records, and in no way compares with the larger subtractive effect which is more usual. Other nerves, though themselves able to promote very fair reflex tension, subtract from the large reflex elicited by the musculo-spiral nerve, that is, they inhibit a part of the big group of motor neurones already activated by the musculo-spiral nerve. It was found that *M. brachialis*, activated by a stimulus of 9 cm. (class coil) to the ipsilateral musculo-spiral nerve, reached a smooth plateau tension of 0.5 kg. A stimulus to the ipsilateral internal cutaneous nerve, itself capable of producing a tension of 0.09 kg. in the resting muscle, when added during the plateau of the reflex from musculo-spiral had no apparent effect. If now the stimulus to the musculo-spiral nerve weakened to 13 cm. the response reached the same tension plateau as before, but the intercurrent stimulus now produced an inhibition of the musculo-spiral response to 0.05 kg. (Fig. 6). Following the work of

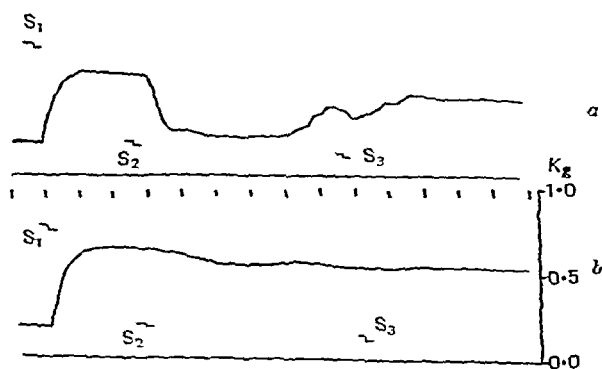


Fig. 6a *Brachialis anticus* Stim. of ipsilat. musculo-spiral nerve, coreless class coil 13 cm. 48 break shocks per sec., from  $S_1$ , with intercurrent stim. of ipsilat. int. cutaneous nerve, Berne coil coreless 11 cm., 50 break shocks per sec., between  $S_2$  and  $S_3$ . Time 0.1 sec

Fig. 6a Similar, but stim. of musculo-spiral nerve strengthened to 9 cm.

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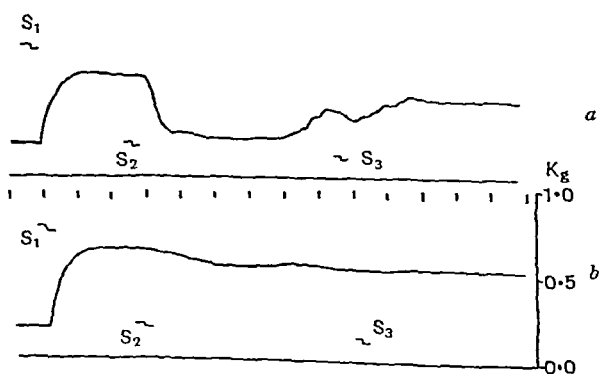


Fig. 6 a. *Brachialis anticus* Stim. of ipsilat. musculo-spiral nerve, coreless class coil 13 cm., 48 break-shocks per sec., from  $S_1$ , with intercurrent stim. of ipsilat. int. cutaneous nerve, Berne coil coreless 11 cm., 50 break-shocks per sec., between  $S_2$  and  $S_3$ . Time 0.1 sec.

Fig. 6 a. Similar, but stim. of musculo-spiral nerve strengthened to 9 cm.

Cooper, Denny-Brown and Sherrington<sup>(3)</sup> we consider that the responses from the internal cutaneous nerve were totally occluded by that from musculo-spiral in both cases. The stronger musculo-spiral stimulus was not able to produce any higher tension than that evoked by the weaker stimulus, because the latter already evoked activation in the total number of final motor units available to musculo-spiral. The weaker stimulus effected this without activation of all the musculo-spiral afferent nerve fibres, so that those remaining, when they were also activated by the stronger stimulus, effected a change in the susceptibility of the motor units to ipsilateral inhibition. In order that this should occur it must be supposed that these higher threshold afferents play on motor units already activated by the lower threshold afferents. It was shown by Cooper, Denny-Brown and Sherrington that there is considerable occlusion between small branches of lower limb afferents such as the popliteal nerve, and the same process is here at play in the nerves of the fore-limb. The musculo-spiral nerve on account of its enormous homogeneous mutually overlapping flexor exciting component thus has, when excited maximally, the prepotent extensor inhibition and flexor excitation mentioned above. Further evidence of the cumulative excitatory effect produced by nerve trunk stimulation is provided by the observation that an ipsilateral stimulus may inhibit the after-discharge of *M. brachialis anticus* although it is without effect during the excitatory background stimulus.

Reference has been made to the unexpected influence of *M. biceps brachii* which when stretched produces an inhibitory fall in its fellow muscle *M. brachialis anticus*. Some experiments therefore were made in furtherance of this observation. It will be recalled that in the cat the muscle has in the majority of animals only one head (tendinous) of origin, that from the supraglenoid tubercle. The insertion (tendinous) as in man is to the radius. It is therefore a "double-joint" muscle and one moreover which is peculiarly well fitted for myographic investigation by reason of its narrow tendinous origin and insertion. We had tacitly assumed *M. biceps brachii* to be a typical flexor of the elbow. It has been surprising to find that the muscle shows many of the reflex activities which are associated with extensor muscles, especially *M. supraspinatus*. For instance, stimulation of a nerve of the opposite fore-limb produces in the muscle an abrupt jet-like onset, followed by a rapid slight fall and then a slower ascent, though on occasion the abrupt onset precedes indistinguishably the main ascent, types of tension development which are not uncommon with extensor muscles. Such contractions can be

inhibited in large part by the ipsilateral internal cutaneous nerve to a very low tension (0.01 kg.) corresponding to the small contraction elicited by the ipsilateral nerve alone. Other nerves of the contralateral fore-limb have also the effect of producing powerful contractions in the muscle. Although these "crossed" nerves are not without excitatory action also on *M. brachialis anticus*, the degree of tension there is very small indeed (*e.g.* 0.2 kg. or less, as a general rule). The high tensions promotable in *M. biceps brachii* from stimulation of a crossed nerve (*e.g.* 0.95 kg., Fig. 7) at once place it in a position suggestive rather of

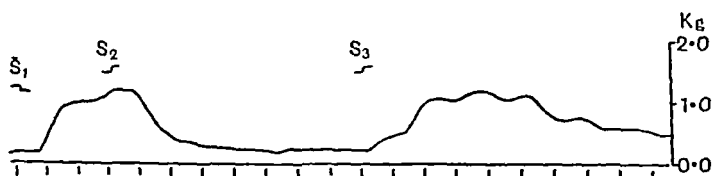


Fig. 7. *Biceps brachii*. Stim. of contralateral ulnar nerve, Berne coil coreless 12 cm., 50 break-shocks per sec., after  $S_1$ , with intercurrent stim. of ipsilat. int. cutaneous nerve, class coil coreless 8.5 cm., 48 break-shocks per sec., between  $S_2$  and  $S_3$ . Time 0.1 sec.

an extensor type of muscle than of a flexor type. Another feature which distinguishes *M. biceps brachii* from most flexor muscles so far investigated is in the facility with which a reflex tension in response to stretch of the tendon (stretch-reflex) can be elicited (Fig. 8). Although a response

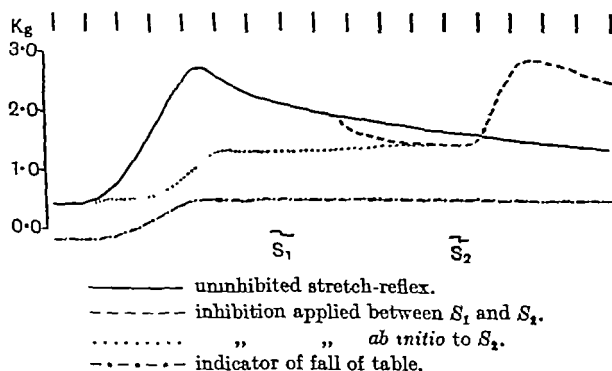


Fig. 8. *Biceps brachii*. Stretch-reflex.

- (i) Control stretch of 4 mm. to tendon in all records.
- (ii) Inhibitory stim. of ipsilat. nerve to latissimus dorsi applied between  $S_1$  and  $S_2$ , Berne coil coreless, 50 break-shocks per sec., 13.5 cm.
- (iii) Same stim. applied *ab initio* to  $S_2$ , after which myographic tracing corresponds with that of (ii). Time 0.1 sec.



to quick stretch (pluck or tap) is not uncommon in flexor muscles (1, 5) (and it is found in *M. biceps brachii*) it has not, so far as we are aware, been noted that the development and maintenance of a considerable reflex tension ensue on the application of a slow stretch (4 mm. during 0.36 sec. to a muscle of total length 9.0 cm.), even with an intercollicular section of the brain stem. Hitherto, such slow stretches applied to muscle tendons have, in their peripheral recruitment of stretch organs, been effective to elicit and maintain high degrees of reflex tension only in typical anti-gravity (extensor) muscles. This stretch-reflex of biceps can be inhibited *ab initio* by the ipsilateral nerve to *M. latissimus dorsi* producing a tracing of passive tension which corresponds to that of the paralysed muscle—an observation in conformation with the usual behaviour of anti-gravity muscles in response to stretch.

Ipsilateral nerves, however, need not be always inhibitors with regard to *M. biceps brachii* but may elicit a gradual development of tension suggestive of a "recruitment" of motor neurones in the centre. The type of curve is often as gradual as that elicited by stimulation of the contralateral nerve and resembles the response of *M. supraspinatus* to stimulation of the median nerve at the wrist rather than that of a flexor muscle responding to an ipsilateral stimulus. On occasion, there has, on the other hand, been striking resemblance of the contralateral response to the ipsilateral in the abruptness of onset and smoother secondary rise—a feature suggestive of a flexor's activity followed by an extensor's. Although we have made fewer observations on *biceps brachii* than we have on *M. supraspinatus*, we have obtained a number of records in which the rise of tension in biceps in response to stimulation of the ipsilateral ulnar nerve is so closely similar to the recruitment rise of the typical extensor, *M. vastocruceus*, that the record is indistinguishable except after close examination (Fig. 9 a). The point of difference is an interesting one, and is more clear in the string record than in the myograph record. It is that, on withdrawal of the stimulus, the string vibrations soon cease entirely (within  $40\sigma$ ) while the myograph record shows  $10\sigma$  later a rather sharp turn on to the decline of a diminished after-discharge<sup>1</sup>. This relatively abrupt fall has been observed in preparations in which the ipsilateral median nerve produced a similar recruiting response and prolonged after-discharge without any abrupt turn accompanied by string vibrations of considerable magnitude and frequency. Nevertheless, there are records showing that although either

<sup>1</sup> A similar type of response has been noted from *supraspinatus* stimulated by median at the wrist (p. 307).

of these two ipsilateral nerves may produce a recruiting rise of tension in biceps, the withdrawal of the stimulus is accompanied by more or less

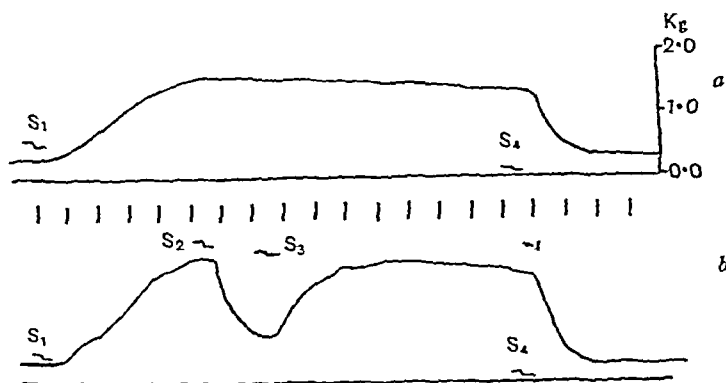


Fig. 9a. Biceps brachii. Stim. of ipsilat. ulnar nerve, Berne coil coreless 12 cm., 50 break-shocks per sec., between  $S_1$  and  $S_4$ . String record not reproduced.

Fig. 9b. Similar, with intercurrent stim. of ipsilat. int. cut., coreless class coil 8 cm., 48 break-shocks per sec., between  $S_2$  and  $S_3$ . Time 0.1 sec.

rebound—clear evidence of a concomitant inhibition during stimulation. An ipsilateral nerve which is as powerful an inhibitor of biceps in ipsilateral as in crossed reflexes is the internal cutaneous (Fig. 9b). The type of inhibition is the same in both cases. This behaviour of biceps in reflex relation to the internal cutaneous nerve is one example of its similarity to brachialis anticus.

These preliminary observations, however, on biceps brachii show that of its reactions, a moiety is extensor-like rather than flexor-like.

#### DISCUSSION AND CONCLUSION.

In the fore-limb there can commonly be found departure from the rule which obtains largely in the hind-limb that electrical stimulation of a nerve of the limb promotes withdrawal ("flexion") on the same side. Although in the hind-limb a small degree of extension can be obtained by stimulation of an ipsilateral nerve the tension developed by extensor's muscles under those conditions is slight in comparison with that registered when a nerve of the opposite hind-limb is stimulated (crossed extensor reflex). In a muscle of the fore-limb (e.g. *M. supraspinatus*), however, we have recorded tensions of e.g. 0.75 kg. promoted by stimulation of the small ipsilateral digital nerve in a preparation in which stimulation of a contralateral nerve trunk (whole median) produced a tension of 1.5 kg.,

that is, only twice as many final motor neurones, approximately. There is little difference in the general character or degree of tension in response to ipsilateral stimulation whether the branch of the median nerve be stimulated at the wrist or one of its twigs in the digital clefts. The slender digital nerves in the external border of the paw, therefore, are powerful promoters of ipsilateral extension and each must have, also, a widespread ramification among the final motor neurones of *M. supraspinatus*, and one that overlaps considerably with the distribution of its fellows. Activation of *M. supraspinatus* can be brought about too by stimulating the skin of the pre-axial (radial) border of the forearm. (In certain clinical cases, Riddoch and Buzzard have similarly recorded extension of the arm in response to nociceptive stimuli of the hand (10a).) On the contrary, the ulnar nerve has an action which is preponderantly one of inhibition in this reflex as in other extensor reflexes. The nerve itself also or its digital branches are almost equally efficient for the purpose, a fact which again points to a wide distribution among and an intimate association with the final motor neurones of the muscles. Stimulated by itself it produces only a small degree of excitation in the muscle recalling the reflex similarly elicited in a hind-limb extensor, and to the same degree of tension as a general rule, though rarely this may be surpassed. In keeping with the reflex outcome of exciting this nerve, stimulation of the skin of the post-axial (ulnar) border of the forearm is followed by inhibition of *M. supraspinatus*. This high degree of unit representation of digital nerves at spinal centres has been previously noted, especially for the characteristic of promoting flexion of a limb response to a nociceptive stimulus (3).

The well-known "extensor thrust" of chronic spinal preparations is a response to mild mechanical pressure on the ball of the foot (11). The brief nature of the extensor thrust suggests that the central discharge from the motor neurones consists of a brief volley of impulses without any post-stimulatory volleys to maintain a postural plateau. This lack of posture is correspondingly absent from the tendon jerk of *M. vastocrureus* in the spinal preparation. Nevertheless, this "extensor thrust," as well as the extension elicited from a limb on application of rheonomic currents to an ipsilateral nerve (12) and the *stützreaktion* in thalamic or decerebellated animals in response to natural mechanical stimuli of the limb (2, 14, 15) all show that there exist nerve paths which are capable of exciting extensor muscles in the ipsilateral limb. In our experiments these paths have been stimulated with repetitive electrical stimuli (tetanic) and have activated motor neurones of an indubitable anti-

gravity muscle of the ipsilateral limb, *M. supraspinatus*. Whether or not this is a kinetic extension, as in the extensor phase of the limb in progression, or is an aspect of static extension, that is, passive non-motile resistance to flexion resulting in fixation of the limb (*stützreaktion*), it is not possible to say at present. The reflex extension in response to a stimulus to the same limb appears to have its afferent focus at the apex of the limb. Although our method of experiment has not enabled us to determine definitely whether the afferent area is under normal circumstances purely a cutaneous one, the majority of evidence suggests that both cutaneous and muscular afferents take part. Under certain conditions (*e.g.* after hæmorrhage) the afferent field will spread proximally to include afferents of definitely muscular origin (6).

In the hind-limb of spinal preparations Cooper, Denny-Brown and Sherrington found an ipsilateral flexor inhibiting and extensor exciting reflex elicitable from the popliteal nerve and in slight degree from the peroneal. Our observations show that this extension producing reflex is present in the fore-limb in the same manner as in the hind-limb, and we should suggest that this tetanic response, the extensor thrust, and the positive supporting reaction (*stützreaktion*) are all phases of the activity of the same reflex mechanism under different conditions.

*Biceps brachii*. Flexion as a motor response to stimulation of the cerebral cortex is more easily elicited than extension. In the experience of one of us (D. E. D.-B.) biceps is peculiar among "flexors" for being much more difficult than brachialis anticus to excite from the motor cortex of the monkey. That an important function of the biceps brachii in man is flexion of the elbow cannot be called into question. In the cat also it is evident that the muscle can act as a flexor, although the myographic record obtained by stimulation of an ipsilateral nerve has not always the usual flexor form with abrupt ascent and rapid turn to the plateau. A marked deviation from the usual flexor-like behaviour towards extensor-like behaviour is found in the facility with which quite large contractions of long latent period and slow ascent are elicited on stimulation of a nerve of the contralateral limb, that is, of the "crossed extensor" recruitment type (7). An even more interesting peculiarity of the biceps is the presence of a well-marked and well-maintained stretch-reflex occurring in preparations in which the level of section of the brain stem is intercollicular. The stretch-reflex as in typical extensor muscles can be reflexly inhibited to a level of tension which is but little above the tension of passive stretch or can be abolished by section of the muscle's nerve. This surprising property of responding to maintained

mechanical stretch by a maintained active reflex tension has its simpler expression in the response to small brief stretches (*e.g.* taps on the tendon) by "jerks," which is however no peculiarity of extensor muscles only but is a well-known property of flexors, under the same conditions of experiment. If the preparation had been "thalamic," it would be less surprising to find a maintained stretch-reflex in a flexor muscle, since from clinical observation this would be expected, its existence having indeed already been accepted by some investigators.

It is well known that "flexor tone" of the fore-limb may occur in preparations decerebrated at the intercollicular level by the trephine method when the preparations are not in good condition, as well as in preparations made by the anæmic method (9, 10). Although this may have occurred in our experiments, we are of the opinion that the stretch-reflex of biceps is most easily demonstrated when the preparation, as judged by the state of the hind-limbs and supraspinatus, shows well-marked extensor rigidity. We have not found the stretch-reflex altered in any way in the thalamic preparation. We are then in agreement with Schoen (15) that the biceps brachii muscle can operate as an anti-gravity muscle—and that can only be at the shoulder joint.

#### SUMMARY.

1. The median nerve at the wrist, stimulated tetanically, evokes a reflex contraction in an ipsilateral extensor (*M. supraspinatus*) of considerable tension (0.7 kg.). The development of tension has a gradual onset.

2. The forearm branch of the median nerve inhibits the reflex, as does stretching of *M. flexor digitorum profundus*.

3. The branches of the median nerve in the digital clefts excite the reflex to high degree.

4. The ulnar nerve and its branches in the digital clefts inhibit the reflex.

5. *Brachialis anticus* in response to nerve stimulation behaves as a flexor muscle. Its reactions show that there is considerable excitatory overlap in the nerve centres and only a small degree of inhibitory overlap. The ipsilateral internal cutaneous nerve has an inhibitory action.

6. *Biceps brachii* is not a typical flexor, as it shows marked response to stimulation of a nerve of the opposite fore-limb as well as a maintained stretch-reflex. In these respects it is extensor-like.

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## TABULAR APPENDIX.

+ = weak excitation.  
 + + = moderate excitation.  
 + + + = strong excitation.  
 + + + + = very strong excitation.  
 - = inhibition, similarly.

The presence of brackets denotes effect when played against the background of a pre-existing reflex excitation.

The absence of brackets denotes effect of the nerve when stimulated alone.

	Ipsilateral nerves					
	Median in arm	Median in forearm	Median at wrist	Ulnar in arm	Ulnar at wrist	Int. Cut.
M. brachialis anticus	+ + + (-)	+ +	- - - (+ +)	+ + + +		+ (- - -) (+)
M. biceps brachii	+ (-)	+	+ + (+)	+ +		+ (- - -)
M. supra-spinatus	variable	(- - -)	+ + + (-)	+ (- - -)	(- -)	+ (- - -)
	Musc. spiral (whole)	Circum-flex	Nerve to latissimus	Sub-scapular nerve	Musc. Cut. nerve to brachialis	Musc. Cut. nerve to biceps
M. brachialis anticus	+ + + +	+ (-)	+ (- -)	+ (-)		+ (- -)
M. biceps brachii	+ (-)		+ (- - -)	+ -	+ (- -)	
M. supra-spinatus	- - -	- -	+	-	+ + (-)	+ (- - -)
	Contralateral nerves					
	Median in arm	Ulnar in arm	Int. Cut.	Musc. spiral		
M. brachialis anticus	+	- -		+ +		
M. biceps brachii	+ +	~ + + +	+ +			
M. supra-spinatus	+ + + +	+ + + +	+ + + +	+ + + + (-)		

*N.B.* This table is composed on an assessment of the average results of 45 experiments. Individual peculiarities and deviations are not expressible within the compass of the table.

# FURTHER OBSERVATIONS ON THE VASO-MOTOR REFLEXES AND ASSOCIATED PHENOMENA.

BY SWALE VINCENT AND J. H. THOMPSON.

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## INTRODUCTION.

THE text-books give a meagre and, in our opinion, an unsatisfactory account of the vaso-motor reflexes. It is usually assumed that sensory nerves are pressor nerves, and that, when, for any reason, they act as depressor nerves, this is to be regarded as an exceptional "reversal" of their true and proper action. This, we consider, is an unjustifiable attitude to adopt. The older workers usually employed two or three different kinds of anæsthetics, and very commonly administered curare and even atropine in addition. It is now recognised by many investigators that the results obtained from experiments on animals under anæsthetics may differ in many respects from those on the unanæsthetised (pithed or decerebrate) subjects. Thus, Macdonald and Schlapp<sup>(1)</sup> have shown that the depressor effect of small doses of adrenaline is due to a pharmacodynamical influence of the anæsthetic. This has recently been confirmed by Vincent and Curtis<sup>(2)</sup> who find also that the shape of the splanchnic blood-pressure curve depends on the presence or absence of certain anæsthetics. These considerations have prompted us to a re-investigation of the vaso-motor reflexes in the decerebrate animal.

Very little attention has been paid to blood-pressure reflexes brought about by stimulation of the terminals of sensory nerves. Previous to the work of Vincent and Cameron<sup>(3)</sup>, and Ogata and Vincent<sup>(4)</sup> we can find no records of experiments bearing upon this point.

It is shown that in the normal animal the reflexes under discussion occur as a result of stimulation of the skin and other sensory surfaces, or from muscles, somatic or splanchnic. We have so far confined our investigations to large and small nerve trunks, the intestine, the skin, and skeletal muscles.



## LITERATURE.

The literature of the subject up to 1919 will be found in the papers of Vincent and Cameron(3), and of Ogata and Vincent(4). These observers paid special attention to the effects of various anæsthetics when given singly or in combination. They found as others had found that in the usual laboratory animals when every allowance is made for the effect of anæsthetics, a general law can be enunciated to the effect that a strong stimulus will produce a reflex rise of blood-pressure while a weak one will give a fall. The frequency of stimulations has also an effect. With a frequent rate of stimulation a rise is obtained, and with an infrequent rate a fall (Gruber(5)). Cooling the nerve tends to produce vaso-dilator effects (Howell(6) and others). Stimulations of a recently regenerated nerve tends to produce a reflex fall of pressure (Reid Hunt(7)). Vincent and Cameron were the first, it would appear, to investigate the result of stimulating nerve terminations, such as those in the skin, muscle and the intestine. They found that stimulation of the skin, kneading of muscle, and manipulation of the intestine all cause a fall of blood-pressure under certain conditions and a rise under other conditions. Ogata and Vincent thought that violent or extensive stimulations of the skin produce a rise while weaker stimulation causes a fall. As will be seen, we cannot confirm this.

## METHODS.

(a) *General.*

All the experiments have been performed upon decerebrate cats. After the preliminary operations of inserting a tracheal tube and tying both carotid arteries (the animal being under ether anæsthesia) decerebration was performed by means of a small trephine hole in the side of the skull. This was enlarged by bone forceps. By this method less bleeding results than by a large trephine hole in the top of the skull and, moreover, a clear view of the plane of transection can be obtained when the fore part of the brain is removed. The brain stem was transected usually between the anterior and posterior corpora quadrigemina and the cranial cavity carefully packed with small swabs of cotton wool soaked in warm normal saline.

The blood-pressure was recorded in the usual way by means of a mercurial manometer connected with one of the carotid arteries.

In all cases at least one hour was allowed to elapse after decerebration before any stimulation in order to obviate the effects of ether anæsthesia.

In the majority of cases normal respiration was continued and a record taken.

(b) *Special.*

*Nerve.* Small skin nerves in the region of the thigh were dissected out and stimulated by an induced current at an approximately constant frequency. Small bundles of the sciatic nerve were also separated carefully from the main trunk and stimulated in a similar manner.

*Intestine.* Stimulation took the form of a kneading manipulation by the hands, care being taken to avoid sustained pressure and pulling on the mesentery.

*Skin.* Scratching of the skin was performed with blunt scalpels, and the stimulation was varied by gradation of the intensity and extensiveness of the stimulus. The intensity of the stimulus was increased by more vigorous scratching over the same area but was never sufficient to draw blood, and the extensiveness of the stimulus was varied by the employment of several scalpels over different areas at the same time. Various parts of the body surface were used, including arms, legs, thorax, abdomen and ears.

*Muscle.* The experiments were performed upon the gastrocnemii muscles. The tendo achillis was cut close to its insertion, and the muscle separated from the leg as far as its origin. The popliteal vein and the sciatic nerve were exposed in the popliteal space. It was thus possible to knead the muscle without stimulating adjacent structures. Kneading was performed in a manner comparable to that applied to the intestine.

## RESULTS.

### *The nerves.*

*Variations in the strength and frequency of the current.* Using the decerebrate animal we find that strong stimulation of the main trunk of the sciatic nerve gives a rise of blood-pressure, while a weak one will give a fall. These results are independent of alterations in the respiratory rhythm or depth, as was shown by a simultaneous record of the respiratory movements. We have also stimulated the sciatic nerve with the same strength of current, but with different rates of interruption of the primary circuit, and find that a rise is obtained with the rapidly interrupted stimuli, and a fall with interruptions of less frequency (Fig. 1). This result was first obtained by Gruber<sup>(5)</sup> and confirmed by Vincent and Cameron<sup>(3)</sup> and Ogata and Vincent<sup>(4)</sup>.

These experiments show then that the "strong and weak" and the

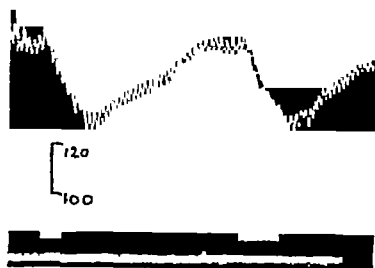


Fig. 1.

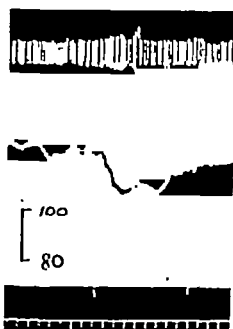


Fig. 2.

Fig. 1. Stimulations of main trunk of sciatic nerve. Strong stimulus. Frequency 4 per second. There are marked falls of pressure.

Fig. 2. Stimulation of small skin nerve. Secondary coil 7 cm. from the primary coil. The effect is a pure fall. Contrast Fig. 4.

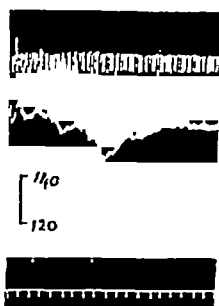


Fig. 3.

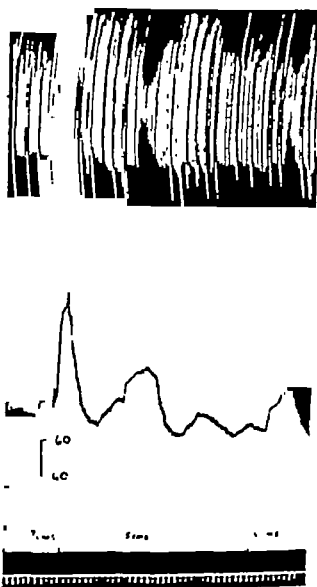


Fig. 4.

Fig. 3. Stimulation of small skin nerve. Secondary coil 15 cm. from the primary coil. Again a pure fall. Contrast Fig. 4.

Fig. 4. Stimulation of main trunk of the sciatic. Secondary coil at 7 cm., 15 cm., 15 cm. respectively from the primary coil. Same rapid frequency as in Figs. 2 and 3. Marked rises of pressure are now obtained instead of falls.

"frequent and infrequent" laws hold good even in an animal without any anæsthetic.

*Variations in the size of the nerves stimulated.* On several occasions falls of blood-pressure were obtained upon stimulating small skin nerves or small bundles of the sciatic nerve (Figs. 2 and 3), and when the same stimuli were applied to the sciatic nerve, rises of blood-pressure were produced (Fig. 4). Such results have not been obtained invariably, and this we attribute to the difficulty of securing such small nerves undamaged and of maintaining a constant E.M.F. in the secondary circuit of the apparatus used.

### *The intestine.*

(A) *Stimulation of the intestines by kneading for 10 sec. with all the nerves intact.* In every case an initial rise of blood-pressure was recorded and the remainder of the results of each stimulation (consisting of either a secondary rise or a fall or both) depended upon (1) the amount of the exposure of the gut, (2) the period of exposure, and (3) the number of successively repeated stimulations.

1. We kneaded the gut whilst it was still in the abdominal cavity, and found that a marked secondary rise occurred invariably. The

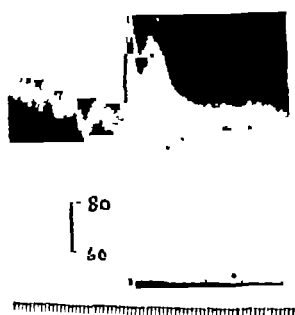


Fig. 5.

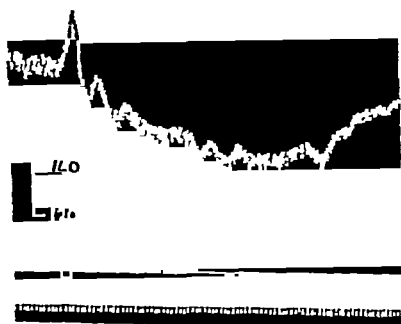


Fig. 6.

Fig. 5. Kneading intestine (whilst still in the abdominal cavity) through mid-line incision gives a rise without any fall. Note the marked secondary rise.

Fig. 6. Artificial respiration. Kneading of intestine applied after prolonged exposure. Marked after fall of pressure.

secondary rise took place shortly after the initial rise had begun to pass off (Fig. 5).

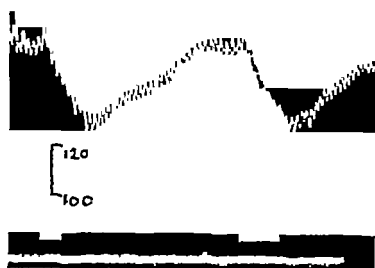


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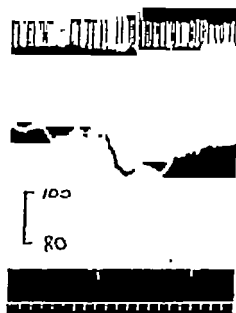


Fig. 2.

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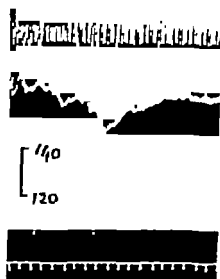


Fig. 3.

Fig. 3. Stimulation of small skin nerve. Secondary coil 15 cm. from the primary coil. Again a pure fall. Contrast Fig. 4.

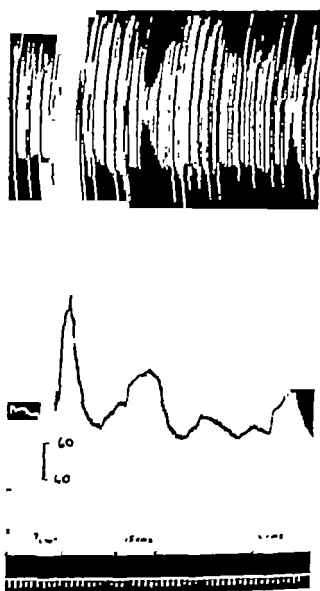


Fig. 4.

Fig. 4. Stimulation of main trunk of the sciatic. Secondary coil at 7 cm., 15 cm., 15 cm. respectively from the primary coil. Same rapid frequency as in Figs. 2 and 3. Marked rises of pressure are now obtained instead of falls.

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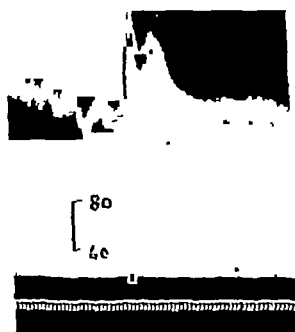


Fig. 5.

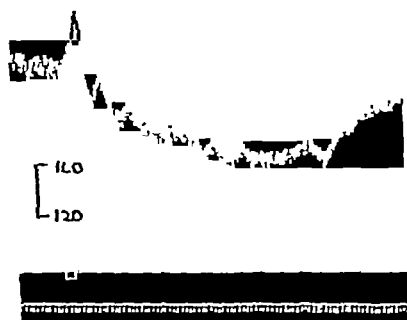


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secondary rise took place shortly after the initial rise had begun to pass off (Fig. 5).

After the intestines had been taken out of the abdominal cavity and freely exposed, similar stimulations caused, in the great majority of cases, an after fall in addition to the initial rise and secondary rise.

2. Moreover, this after fall shows a definite increase in depth below the original level as the period of exposure lengthens (confirmatory of Vincent and Cameron) (Fig. 6). We found this to be so in all our experiments. Often the first stimulation after general exposure of the gut—provided it was applied *immediately* after general exposure—revealed only a small fall, and on a few occasions failed to produce any fall at all. But each successive stimulation thereafter increased the fall, and it was initiated by the second stimulation when the first failed to produce it (Fig. 7). We shall show that this apparent “failure to fall”

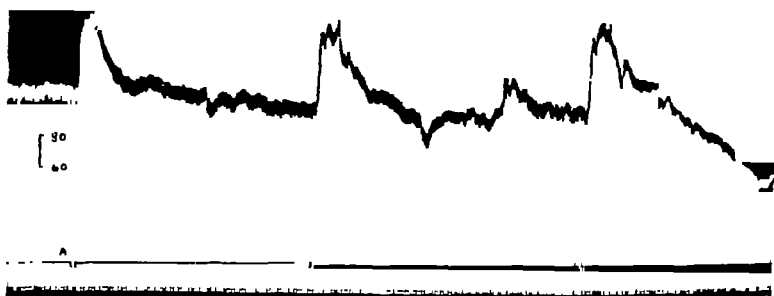


Fig. 7. Effects of successive kneadings of intestine. The first stimulus (A) was applied immediately after displacement of the intestines from the abdomen. Note the gradual production of an after fall and the descent of the position of the secondary rise on the decline of the initial rise.

in these instances is quite compatible with the conclusions we have reached.

Another effect was observed upon the secondary rise after general exposure of the gut. Just as the fall deepened with the length of exposure, so did the secondary rise appear lower and smaller on the decline following the initial rise, until finally it disappeared in the fall.

3. A further factor influencing the results was the effect of successive stimulations at short intervals. In all such series of experiments the initial rise was gradually diminished and the secondary rise disappeared, in a manner corresponding to that indicated above, and eventually was replaced by a distinct fall.

(B) *Stimulation of the intestines by the insertion of cotton wool plugs and balloons, all the nerves remaining intact.* We performed several

experiments in which we used as a stimulus a plug of cotton wool, which was passed backwards and forwards within a loop of small intestine at a rate of 2 excursions per sec. (approx.); we also incised a portion of gut longitudinally and rubbed the mucous membrane with a similar plug. A small balloon was passed into the lumen and inflated for periods of 10 sec. and 20 sec., and other experiments were performed in which the balloon was inflated at intervals of 1 per sec. Results obtained by these methods of stimulation were comparable with those obtained by kneading the gut, the only difference being quantitative.

(C) *Stimulation of the intestines by kneading for 10 sec. with the semi-lunar ganglia extirpated and the vagi cut.* Careful dissection having revealed a lesser splanchnic nerve in the cat, we took the precaution of removing the semi-lunar ganglia in order to eliminate all nervous influence. The vagi were cut in the neck.



As in previous experiments we found an initial rise a constant factor. The secondary rise was present in 83 p.c., and relatively to the initial rise was greater than in experiments under (A) and (B) (Fig. 8).



Fig. 8. Semi-lunar ganglia extirpated. Vagi cut. Abolition of the fall and production of very large secondary rise.

We also found that no after fall was produced, and that the secondary rise was increased by prolonged stimulation. A series of stimulations at short intervals resulted in the gradual disappearance of the secondary rise, but no fall could be obtained at the end of the series as in (A) 3.

### *The skin.*

The results we have obtained may be divided into two main categories:

- (a) Those in which a pressor response was elicited.
- (b) Those in which a depressor response was elicited.

(A) *The "pressor" group.* The rise of blood-pressure resulting from scratching the skin, although varying quantitatively in proportion to the extent of the area scratched and the intensity of the stimulus, showed a similar curve in all cases. The curve consisted of a moderately sharp rise whose maximum height persisted until the end of stimulation, and a decline to the original level composed of two parts, (1) a short quick fall commencing at the end of stimulation, and (2) a distinct—and sometimes pronounced—step inaugurating a slow and gradual return to



the mean blood-pressure (Fig. 9). The sharp rise and fall were either diminished or abolished by avoiding pressure and stimulation of the underlying and adjacent structures, and a delayed and gradual rise with rounded peak and slow decline was produced (Fig. 10).

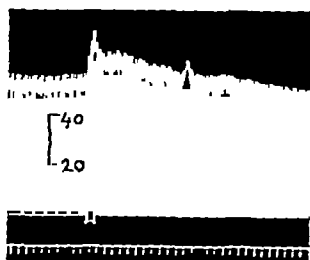


Fig. 9.

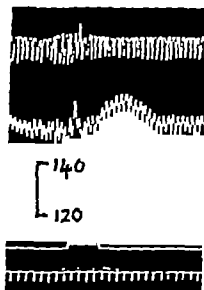


Fig. 10.

Fig. 9. Scratching of skin over abdomen. Note the prolonged decline to the mean level.

Fig. 10. Stimulation of skin of leg by scratching with blunt scalpel. Slight initial rise followed large secondary.

By repeatedly stimulating one area of skin the rise of blood-pressure was gradually diminished and finally abolished.

(B) *The "depressor" group.* The fall of blood-pressure was uncomplicated by any secondary effects shown on the curve. A rapid fall began at the beginning of stimulation, and continued until the end of it. The return to the original level began as soon as stimulation ceased, and the gradient was somewhat less than that of the decline (Fig. 11).

In several experiments in which a rise of blood-pressure was obtained, we noted an obvious tendency for a fall to take the place of the rise in the later stages of the experiments. This was especially noticeable after repeated stimulation. After a period of rest the rise returned.

In one animal no effects upon the blood-pressure could be produced during the period of stimulation. Immediately following the cessation of stimulation, however, a considerable rise of blood-pressure occurred. Later in the experiment similar stimulation caused an initial fall of

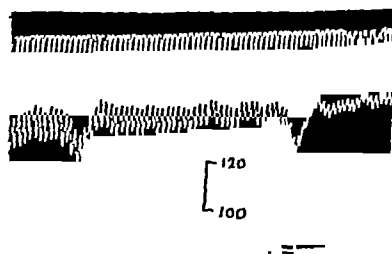


Fig. 11. Effect of scratching skin of right leg. Initial fall. No increase of respiration.

pressure, which commenced at the *beginning* of stimulation and was followed by a secondary rise. Still later, falls were registered without secondary rises. Administration of chloralose markedly increased the depth of the fall (Fig. 12).



Fig. 12.

Fig. 12. 10 c.c. chloralose saturated at 40° C. administered intravenously 5 min. before application of stimulus. Extensive stimulation over both arms and legs. Very marked fall.

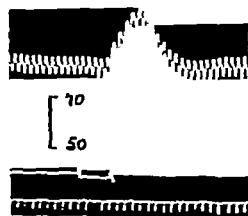


Fig. 13.

Fig. 13. Kneading of left gastrocnemius muscle. A pure rise.

### The Muscles.

As in the case of the results obtained by stimulation of the skin, so with the kneading of the muscles two sets of responses have been observed.

(A) *The "pressor" group.* (1) *Stimulation of the intact muscle.* There was a noticeable tendency for the initial rise to be omitted in the curve, and in such instances the secondary rise was delayed, and the gradient was much smaller than that obtained in the intestine (Fig. 13). Also, the whole rise had a configuration similar to a secondary rise, *i.e.* a gradual incline and decline with a rounded apex. With very large muscles or groups of muscles the curve corresponded to the curve resulting from scratching a skin area with pressure.

(2) *Stimulation of the denervated muscle.* The sciatic, anterior crural, and obturator (anterior and posterior divisions) nerves were divided. The rise of blood-pressure due to stimulation of the muscle was increased.

(3) *Stimulation of the muscle with veins clamped.* The popliteal and femoral veins were clamped by means of artery clips. The rise was completely obliterated (Fig. 14), and returned when the clips were

removed. A similar experiment was performed on the denervated muscle with identical results.

(4) *Repeated stimulation of the intact muscle* resulted in a rapid decrease in the height of the rise and ultimate failure to give any response. Similar stimulation of the denervated muscle in the same animal required a much longer period of stimulation before the rise could be eliminated.

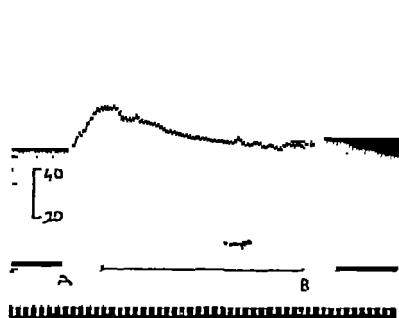


Fig. 14.

Fig. 14. Kneading of left gastrocnemius. *A*, with veins open. Note prolonged decline. *B*, with veins clamped. Blocking the venous return abolishes the rise.

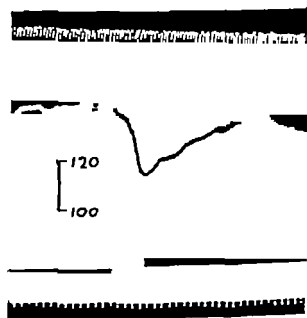


Fig. 15.

Fig. 15. Kneading of left gastrocnemius. A pure fall.

(B) *The "depressor" group.* (1) *Stimulation of the intact muscle.* The fall was rapid and recovery equally so (Fig. 15).

(2) *Stimulation of the denervated muscle.* This resulted in the abolition of the depressor response. In the same animal it was possible to obtain a marked fall with the left gastrocnemius intact, and no response with the right gastrocnemius denervated (Fig. 16).

(3) *Stimulation of the intact muscle with the veins clamped.* No change was effected beyond an exaggeration of the depth of the fall.

In two animals rises were obtained early in the experiment and falls later.

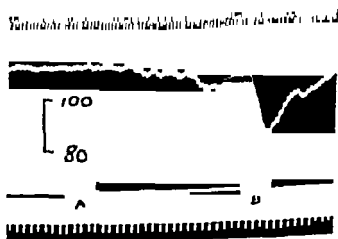


Fig. 16. First stimulation (*A*) applied to denervated right gastrocnemius muscle. Result, nil. Second stimulation (*B*) applied to intact left gastrocnemius muscle. Result, a fall.

*Concomitant phenomena.*

*Respiration.* We found that similar results were obtained under artificial respiration, as with normal respiration. Most of the results were obtained with animals breathing naturally. In some cases falls were accompanied by increased respiration and were probably augmented indirectly thereby (Vincent and Cameron (3)). This chiefly applied to the intestine and the skin; abnormal respiratory effects were rarely recorded in the series of muscle experiments. All experiments, in which increased respiration took place coincident with falls of blood-pressure, have been discarded.

*Peristalsis.* Kneading of the gut produced no increase of peristalsis, and no detectable alteration in the blood-pressure tracings was caused by peristaltic action. We thought that the passage of the plug of cotton wool in an antiperistaltic direction slightly exaggerated the effects of the stimulation upon the blood-pressure.

## DISCUSSION OF THE RESULTS ON NERVE TERMINALS.

Examination of the results shows that three distinct effects upon the blood-pressure may be produced: an initial rise, a secondary rise, and a fall, and that these three effects may be in evidence in any one animal upon stimulation of either the intestine, or the skin, or the muscle.

We consider the initial rise to be due solely to the mechanical effect of the stimulation. The absolute constancy and similarity of its appearance under all conditions in the case of the gut (a very vascular area), combined with the facts that it is only present in the skin stimulation when pressure is exerted upon underlying structures, and in the muscle stimulation when large muscles or groups of muscles are used, warrant this assumption. However deftly the kneading of the gut is done, it is impossible to avoid exerting a little pressure, and the consequent expulsion of blood will be sufficient to explain the form of the initial rise and its constant presence. Herein the decerebrate animal appears to differ from the anæsthetised. Vincent and Cameron found that a marked fall of blood-pressure without an initial rise was almost invariably obtained after the intestines were completely exposed in the anæsthetised animal. Probably the mechanical effect was neutralised by the cause of the fall.

In the case of the fall of blood-pressure, the fact that it could never be obtained in any tissue after all nervous influence had been removed

has led us to the inevitable conclusion that it is due to a true vaso-motor reflex. Such cancellation cannot be effected by interference with the vascular system of the tissue, which, contrariwise, increases the fall. Moreover, where results are not masked by a mechanical effect (skin and muscle), the fall is seen to be a steep one which commences without any delay, and recovery is quick, thus typifying a nervous response rather than a chemical. Repeated stimulation never caused the fall to be abolished. This fall of blood-pressure cannot be ascribed to a reflex causing increased respiration, and hence a fall of blood-pressure due to a mechanical action upon the heart, since all the falls included in the results quoted above occurred without any change in respiration.

The secondary rise cannot be nervous in origin because it still persists after section of all the nerves. In fact, it is present under all conditions except three: (a) after prolonged exposure and manipulation (intestine, muscle), (b) after frequently repeated stimulations (intestine, skin, muscle) and (c) after clamping or ligature of the venous return (muscle). It is important to notice that after frequently repeated stimulations a period of rest results in the reappearance of the secondary rise upon stimulation, and that after removal of the clamps upon the veins a rise of blood-pressure is often to be observed. Obviously, this implies in the former case an exhaustion and a restoration of some influence, and, in the latter, a damming back and subsequent liberation of a similar influence. Series of similar experiments with denervated intestine and muscle gave identical results. We infer from this that the secondary rise is due to the liberation of some chemical substance having a pressor effect, in fact, an autacid pressor substance.

This inference is further corroborated by the form and incidence of the secondary rise in all the experiments. It is most in evidence at the beginning of an experiment when the tissues are fresh. Again, there is always a well-marked delay after stimulation before it appears. Its form is in marked contrast to the fall, the gradient of the incline being small and the return to the mean pressure being very slow and gradual, such as would be expected from the liberation of an autacid substance into the blood stream.

It therefore becomes clear that two mutually antagonistic influences are at work—the vaso-motor reflex causing a fall of blood-pressure, and an autacid substance having a pressor effect. Further examination of the results confirms this. Thus, in the case of the gut when the secondary rise is very marked—as before general exposure of the gut—no fall is recorded; when the secondary rise becomes smaller the fall appears and

increases proportionately to the decrease of the secondary rise. The first stimulation after exposure thus becomes the critical one, and we found that when the secondary rises prior to general exposure were extra large, there was no fall on application of the first stimulus, and when the secondary rises were small prior to general exposure, that there were falls afterwards. Our conclusion that the influence causing the secondary rise antagonised the vaso-motor reflex was also confirmed by the series of experiments when repeated stimulation of the intestine and skin caused the secondary rise to give place to a fall.

Thus, the vaso-motor effect and the chemical effect are mutually antagonistic, and the presence or absence of the secondary rise and the fall, or variation in their relative proportions, is determined by the factors affecting the condition of the tissue. Thus, when the precursor of the autacoid substance is exhausted by frequent stimulation the vaso-motor reflex is manifested; when the vaso-motor reflex is eliminated by removing all nervous control the autacoid substance has unhindered action, and, therefore, we should expect a larger secondary rise under such circumstances. This is what occurs; similarly, denervation of muscle causes the rise of blood-pressure to be greater because it removes the antagonism of the vaso-motor reflex.

Either of these influences may predominate, or, in rare instances, may exactly neutralise each other. Consequently, the individual cat responds to stimulation according to whichever influence is in the ascendency. As our results demonstrate, there are two main groups of animals—those giving a pressor response, and those showing a depressor reaction.

The fact that the stimulation of nerve terminals causes a vaso-motor reflex of a depressor character is quite in harmony with our results obtained by stimulation of nerve trunk. The weak electrical stimulation of the main nerve producing a fall of blood-pressure is possibly equivalent to the most intensive stimulus we have applied to the terminals. Possibly in some instances there is a parallel between stimulation of the terminals and stimulation of a small nerve, which, we strongly suspect, gives a fall of blood-pressure because of the small number of fibres it contains. An adequate conception of the blood-pressure reflexes in the body cannot be obtained by simply stimulating the sciatic nerve with strong induced currents.

## SUMMARY OF RESULTS.

Experiments have been performed upon decerebrate cats after all traces of anæsthetic have disappeared. Stimuli corresponding as nearly as possible to those occurring normally have been applied to the nerves, intestine, skin and muscles under varying conditions. We have found in the case of the nerve that the "strong and weak" and the "frequent and infrequent" laws hold good, and that there is a possibility of the size of the nerve affecting the nature of the response. The results of kneading the intestine show that the amount and period of exposure, the amount of stimulation, and denervation have well-defined effects.

Our conclusions are: that in intestinal, muscular and skin stimulation, two important and antagonistic factors are requisitioned to influence the general blood-pressure; a vaso-motor reflex of a depressor nature, and the liberation of an autacoid substance having a pressor action<sup>1</sup>: that either may predominate, or that the two may neutralise each other: that after exposure, and after prolonged or repeated stimulation, the vaso-motor reflex is in the ascendancy owing to exhaustion of the autacoid pressor substance, and that where very vascular areas are concerned a mechanical effect may be superimposed: that the response to stimulation of various tissues in any one individual may be pressor or depressor according to the general condition and nutrition of the part stimulated and other factors not determined, and that the vaso-motor depressor effect is in harmony with the results obtained by stimulation of nerve trunks.

We beg to acknowledge our indebtedness to Mr M. Kremer for his assistance in conducting some of the preliminary experiments, and to the Ductless Gland Committee of the British Association for a grant to one of us (S. V.).

<sup>1</sup> *Note added in proof.* It is of some interest to recall that in 1903 Vincent and Sheen<sup>(9)</sup> found very definite evidence of the presence of a pressor substance in fresh tissue extracts. It was not found in saline decoctions. We have recently repeated the experiments of Vincent and Sheen and are convinced that such pressor material is undoubtedly present in many fresh tissue extracts, and that it is frequently destroyed by moderate elevation of temperature. Whether this is identical with the autacoid substance described above we cannot yet be certain.

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# THE RÔLE OF THE CORPUS LUTEUM IN THE MAINTENANCE OF PREGNANCY.

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## I. INTRODUCTION.

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*For Insertion in Vol. 64.*

### Erratum. Vol. 64, p. 352.

In the experiments here reported tyramine by misapprehension was used as stated in lieu of ergotamin. Experiments in which the action of ergotoxin was tested are reported in a later paper, this Journ. vol. 65, p. 456.

by Kleinhaus and Schenk(7) for the rabbit and by Daels(8) for the guinea-pig and rat. In the human, the corpus luteum of pregnancy can apparently be removed in the later stages of pregnancy without any effects (Essen-Möller(9), Blair Bell(10)).

There is, however, a large amount of evidence which seems to indicate that the corpus luteum is essential during the whole of gestation, and that its removal even in later stages leads to abortion or reabsorption. This evidence is summarised in Table I.

Analogous data may be obtained from cases where the corpora lutea undergo abnormal degeneration during pregnancy. Hammond(12) and Hartman(20) have shown that foetal death follows such degeneration.

These contradictory results may be partially accounted for by the variation in the methods used to ablate the corpora lutea. Three methods have commonly been used: (a) removal of both ovaries entire, (b) destruction of the corpora lutea by cauterisation, and (c) surgical



TABLE I.

Author	Animal	Method of removal	Result
Blair Bell and Hick (11)	Rabbit	Ovariectomy	Abortion
Hammond (12)	"	"	Abortion or reabsorption
Weymeersch (13)	"	"	"
Drips (14)	Spermophile	"	Abortion
Hess (15)	Cow	Squeezing out	"
Wester (16)	"	Ovariectomy	"
Schmaltz (17)	"	Squeezing out	"
Drummond-Robinson (18)	Goat	Dissection of ovary	"
Hartman (19)	Opossum	Ovariectomy	Reabsorption or abortion

dissection from the rest of the ovary. The first of these has the obvious disadvantage that all ovarian activity is obliterated, not merely that of the luteal tissue. Cauterisation, on the other hand, is difficult to perform with sufficient accuracy to make certain of destroying all luteal tissue. The third method is clearly impracticable in small mammals, and in an animal such as the rabbit, the diffuse nature of the luteal tissue makes its removal most difficult. In the cow, of course, the corpora lutea can easily be shelled out from the ovary without the aid of instruments. In any case it is obvious that the cauterisation or dissection of the ovary constitutes a most severe operation, and one to which no "dummy" manipulation can provide an adequate control. It is difficult, therefore, to say that the observed results may not have been due to operative shock.

Under certain conditions, however, the severity of the operation could be much reduced, and any possible operative shock adequately controlled. In an animal having only one young at a time, and ovulating from one ovary only at œstrus, only one ovary has a functional corpus luteum during pregnancy. The removal of this ovary would eliminate the corpus luteum, therefore, without eliminating other ovarian activity (which would be carried on by the other ovary) and without undue operative shock. Removal of the ovary not containing the corpus luteum would provide a clear control experiment. By using a monotocous mammal pregnant from its first ovulation, the complication of old luteal tissue even could be avoided. Wester(16) notes that abortion took place in a pregnant cow from which the ovary containing the corpus luteum was removed without interference with the other.

A technique has been worked out whereby this condition, naturally occurring in an animal such as the cow, can be experimentally produced in polytocous animals. It has already been shown (Parkes(21)) that exposure to X-rays in the young mouse results in the entire obliteration

of the follicular system of the ovary and in the consequent non-formation of corpora lutea. Such sterilised ovaries are, however, capable of producing œstrus and of performing all ovarian endocrine functions other than those associated with the corpus luteum.

Young mice were therefore sterilised unilaterally, and allowed to become adult, when they possessed one sterilised ovary and one normal ovary going through its normal cycle. The animals were then allowed to become pregnant. In certain of these mice the sound ovary with the corpora lutea was removed during pregnancy, while in others the sterilised one was excised as a control on the operation shock.

This technique, therefore, fulfils the two qualifications which are theoretically desirable, and which have been conspicuously absent in previous work, *i.e.*

(a) The removal of the corpora lutea without eliminating all ovarian activity.

(b) The easy and adequate control of operation shock.

The results of the operations, together with some implications, are described in the present paper.

## II. TECHNIQUE.

*Irradiation.* Unilateral sterilisation of an animal as small as the three weeks old mouse presented certain difficulties. The arrangement finally arrived at was to put the animals in shallow troughs just wide enough to hold them, and about 2 cm. high. These troughs were lined unilaterally with 2 mm. lead, which proved an effective screen to the rays. The dose employed was the same as in previous sterilisation experiments (80 min. of the standard intensity described elsewhere (21, 22)), and to receive this exposure at one time the animals had to remain in the troughs for 2 hours or more. It was therefore necessary to anaesthetise the animals, and this was done with luminal-sodium (.1 c.c. of 1 p.c. solution per 5 grm. wt.). The irradiation was originally given in two halves at intervals of a week, but the increased death-rate from two anaesthetisations led to the total exposure being given at one time.

The animals were so arranged in the troughs that the right ovary was irradiated, while the left was screened and therefore untreated. The rule with these animals was, therefore, right ovary sterilised, left ovary normal. Considering the difficulties attending this technique the results were reasonably good. One hundred and eight animals in all were irradiated unilaterally, and of these only a small proportion failed to show a completely sterilised right ovary on histological examination.

In a still smaller proportion only was the left ovary found to have been affected by the X-rays.

*Operative technique.* Ovariectomy was performed as previously described (23).

*Detection of œstrus, etc.* The detection of œstrus, copulation, and pregnancy was carried out as usual (24). Reabsorption of embryos was usually detected by the presence of large amounts of blood in the smear. At one stage (10–12 days) blood occurs in the smear during normal pregnancy (the “placental sign”), but in this case the smear is highly viscous, whereas the blood smear during reabsorption is fluid.

*Diagnosis of sterility of right ovary.* In a number of animals where the left (fertile) ovary was removed during pregnancy the animal was subsequently mated. Fertility in this case depended on the right (irradiated) ovary and the breeding history of the animal (after left ovariectomy) thus gave some information as to the condition of the right ovary. In every case, however, complete histological examination was made of the right ovary and the presence of even one follicle was considered to constitute fertility. For the experiments in question the presence in the right ovary of corpora lutea atretica or of luteal-like tissue was of importance. Its occurrence was, however, rare. None of the mice upon which left ovariectomy was performed during pregnancy showed atretic or other corpora lutea in the right ovary (except the three described in Table II as fertile). The right ovaries of SM 9 and SM 96 had a somewhat luteal-like appearance (see Brambell, Parkes and Fielding (22)), but any doubt as to the œstrus-producing capacity of these right ovaries is dispelled by the fact that both came into œstrus following the removal of the left ovary.

### III. REMOVAL OF CORPORA LUTEA DURING PREGNANCY.

Twenty-five unilaterally irradiated animals were used for the experiments on the maintenance of pregnancy. All of these were operated upon at various stages of pregnancy between 11 and 17 days. Since pregnancy can only be detected with certainty in the intact animal at 11 days onwards (by placental sign and palpation), it seems inadvisable to perform operations earlier than this stage.

Seventeen of the animals had the fertile left ovary removed, while the remaining eight were used for control operations and had the irradiated right ovary removed. The results of the left ovariectomies are shown in Table II.

TABLE II. Removal of the left (fertile) ovary.

No. of animal	Days pregnant when operated on	Result	No. of foetuses if ascertained	Condition of right ovary	
				Breeding	Histology
SM 8	11	Reabsorption	—	—	Sterile
SM 10	11	"	—	—	"
SM 97	11	"	—	Sterile	"
SM 7	12	"	—	—	"
SM 58	13	Abortion	—	Sterile	"
SM 88	14	"	—	—	"
SM 93	14	"	—	—	"
SM 101	14	"	—	Sterile	"
SM 103	14	Reabsorption	—	—	"
SM 18	15	"	—	—	"
SM 61	15	Abortion	—	Fertile	Partially fertile
SM 86	15	"	—	—	Sterile
SM 91	15	"	7	—	"
SM 102	15	Normal litter	8	—	Completely fertile
SM 104	15	Reabsorption	—	Fertile	Partially fertile
SM 9	16	Abortion	7	—	Sterile
SM 96	17	Full time birth	6 (3 dead)	—	"

In these seventeen animals the right ovary was completely sterilised in all except three cases. In one of these three (SM 102) the right ovary was comparatively normal, while in the other two sterilisation was complete except for the presence of one or two small follicles. As will be seen from the above table the result of removing the fertile left ovary with the corpora lutea was that pregnancy came to a premature conclusion in every case except two. The first exception (SM 102) was the animal in which the right ovary had hardly been touched by the irradiation, with the result that left ovariectomy failed to remove luteal influence. These circumstances entirely explain the discrepancy of SM 102. The second animal in which left ovariectomy was not followed by abnormal termination of gestation was SM 96, which was operated on at 17 days pregnant. Most of the abortions took place two days after the operation, and since the duration of gestation in the mouse is only 19 days, abortion following 2 days after operation at 17 days would be coincident with parturition. Actually this appears to have been the case in SM 96, the removal of luteal influence by operation at 17 days being apparently simultaneous with the time of its normal disappearance at the end of pregnancy. This 2 days' interval found in these experiments between the time of removal of luteal influence and parturition or abortion tallies closely with the interval found previously (25) between the time of injection of oestrin during pregnancy, and the consequent abortion resulting (apparently) from over-riding the corpus luteum.

Summing up these experiments, therefore, it may be emphasised

that in fourteen animals in which the left ovary was removed, and in which the right ovary was completely sterilised, termination of the pregnancy occurred in all cases within two days.

*Control operations.* That the result described above was not brought about by any operation effect was clearly demonstrated by the removal, in eight other animals, of the right (irradiated) ovary, the fertile left containing the corpora lutea being left intact. In no animal did this operation of right ovariectomy produce any result whatever. Table III sums up the control operations.

TABLE III. Removal of right (irradiated) ovary during pregnancy.

No. of animal	Days pregnant when operated	Result	Duration of gestation	Size of litter	Right ovary
SM 46	12	No effect	19 days	9	Sterile
SM 65	12	"	"	3	—
SM 52	13	"	"	4	Sterile
SM 89	14	"	"	2	"
SM 63	15	"	"	5	"
SM 64	15	"	"	8	Fertile
SM 100	15	"	"	5	Sterile
SM 85	16	"	20 days	3	Fertile

The right ovaries of SM 64 and SM 85 contained a small follicle each and must therefore be considered as fertile, but from the point of view of these control experiments this is of no importance. The right ovaries of many of these SM series were very minute, and were correspondingly difficult to deal with surgically. In SM 65 the tissue removed at operation and subsequently examined histologically was found not to contain the ovary, and it is probable, therefore, that the right ovary of this animal was not removed. The uniformity of the other control results, however, is an adequate demonstration of the lack of operative effect and therefore of the validity of the effects obtained on removal of the ovary containing the corpora lutea.

#### IV. DISCUSSION.

The results show conclusively that in the mouse the presence of corpora lutea is necessary during the whole of pregnancy, and that their removal results in the invariable termination of gestation. These experiments, however, differ from those in which double ovariectomy is performed, because the retention of the right sterilised ovary results in the continued presence of œstrin-producing tissue, and it may be that the presence of the œstrus-producing stimulus after ablation of the corpora lutea is a factor in bringing about the termination of pregnancy. This might explain the negative results obtained by some workers for

double ovariectomy, though the majority of workers claim the same results for double ovariectomy as for ablation of the corpora lutea alone. It is possible that experimental interference with the corpus luteum during pregnancy may set into motion the parturition mechanism which apparently is activated by the degeneration of the corpora lutea towards the end of normal pregnancy. Dixon and Marshall<sup>(25)</sup> showed that the ovaries of animals in the immediate pre-parturition condition would yield an extract which, when injected into another animal, such as a bitch, stimulated the posterior pituitary to activity, presumably with a consequent stimulation of the uterus.

In view of the general correlation which exists in rats and mice between parturition and pro-œstrus, it seemed possible that an œstrus-producing stimulus brought about this result, a suggestion which is strengthened by the fact that the injection of œstrin during pregnancy results in the termination of gestation<sup>(25, 27)</sup>. Although further work has not demonstrated that œstrin has any stimulating effect upon the posterior pituitary, it seems that a similarity may exist between (a) the degeneration of the corpora lutea at the end of normal pregnancy, (b) the experimental removal of the corpora lutea during pregnancy (leaving intact the other ovarian functions), and (c) the physiological removal of the corpora lutea on over-riding their dominance by the injection of œstrin.

On Dixon's and Marshall's view the initiation of parturition proper is a positive ovarian act, and is brought about by the secretion by the ovary of a substance that stimulates the pituitary. If this is so, the abortion which follows complete double ovariectomy cannot be compared with premature parturition. In this connection some recent work by Knaus<sup>(28)</sup> is of considerable interest. He finds that the sensitivity of the uterus to pituitrin varies greatly during pregnancy, and that immediately after conception the sensitivity becomes sub-normal and stays below normal during the greater part of pregnancy. At the end of pregnancy, however, this sensitivity returns to normal. Further, Knaus has strong reason for supposing that the decreased susceptibility is due to an action of the corpus luteum. On this view, therefore, the initiation of parturition may depend not on an increased production of pituitrin, but on a sensitivity of the uterus greater than that hitherto found during pregnancy. The degeneration of the corpus luteum at the end of pregnancy (according to Knaus) brings about this result, and is therefore just as much the factor initiating parturition as on the hypothesis put forward by Dixon and Marshall. The interesting

point about this work is that it explains the fact that double ovariectomy may have the same effect as ablation of the corpora lutea only. In the light of Knaus's work, double ovariectomy would permit of the increased sensitivity of the uterus, as well as would the removal of the corpora lutea alone. It is evident, however, that much greater uniformity of experimental results will have to be obtained before it will be possible to decide the precise relationship, if any, between the normal parturition mechanism and that of the abortion which, in certain animals at any rate, follows the removal of the corpora lutea of pregnancy.

#### V. SUMMARY.

1. The determination of the extent to which the presence of the corpus luteum is essential for the normal progress of gestation has been much delayed by the difficulty of ablating the corpora lutea in small animals without removing all ovarian influence by double ovariectomy.

2. By means of unilateral X-ray sterilisation, however, it has been found possible to produce animals with corpora lutea in one ovary only and thus to make possible the experimental elimination of the corpora lutea during pregnancy without interfering with other ovarian activity.

3. Of 17 animals in which the left (fertile) ovary was removed during pregnancy, all except two showed abnormal termination of pregnancy. One of these exceptions was found to have a completely fertile right ovary which had apparently escaped exposure to X-rays, while the other was not operated on until the 17th day of pregnancy.

4. In a group of control animals the right (sterile) ovary was removed during pregnancy, the left (fertile) ovary being untouched. No animal in this group showed abnormal termination of gestation.

5. It is concluded, therefore, that in the mouse the presence of the corpora lutea of pregnancy is essential during pregnancy until their normal retrogression at about the 17th day, and the bearing of this result upon other similar work and upon the theories of the mechanism of parturition is discussed.

My thanks are due to Prof. G. Elliot Smith, F.R.S., by whose permission the irradiations were carried out in the Department of Anatomy of University College.

To Mr H. A. Harris my thanks are due for technical advice.

The animals were drawn from the colony maintained with the aid of a grant from the Medical Research Council, to whom I am indebted for this assistance.

The compensatory hypertrophy of the untreated ovary which follows unilateral sterilisation is being dealt with by Dr F. W. R. Brambell, who has kindly given me information on the histology of the ovaries discussed above.

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# EFFECTS OF VARIATIONS IN INTENSITY AND FREQUENCY ON THE CONTRACTIONS OF THE STOMACH OBTAINED BY STIMULATION OF THE VAGUS NERVE.

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VEACH<sup>(1)</sup> in 1925 investigated the effects of variations in intensity and frequency of the stimulating current on the response of smooth muscle. He concluded that stimulation of the peripheral end of the vagus nerve with currents of relatively low frequencies or intensities had a motor effect on the lower end of the œsophagus, the cardia and the body of the stomach of the cat. Stimulation with considerably higher frequencies or intensities had an inhibitory effect on these structures. He put forward an explanation for the inhibitory effects of currents of high frequency and intensity on the basis of a Wedensky inhibition effect.

Experiments performed on the same lines by other observers have suggested that the response of smooth muscle to stimulation is dependent on the condition of tonus of the muscle. Carlson, Boyd and Pearcy<sup>(2)</sup>, working on cat's cardia, and McCrea, McSwiney and Stopford<sup>(3)</sup>, working on the intact stomach of the cat and dog, have found that stimulation of the vagus nerve elicited a response in the stomach which depended on the existing tonus condition in that organ.

These different responses are explained by Veach on the basis that tonus is dependent upon the passage of propagated disturbances over a peripheral part of the neuromuscular mechanism, and the degree of tonus upon the frequency of these propagated disturbances. Thus, stimulation of the vagus, when the tonus is high, will increase the frequency of the propagated disturbances to an inhibitory value. On the other hand, when the tonus is low, the frequency of the existing propagated disturbances in the peripheral mechanism is also low; stimulation of the vagus will then only raise the frequency of the propagated disturbances to an excitatory value.

These experiments were carried out to investigate the relation between the response of the stomach to different types of vagal stimulation and the condition of tonus in the smooth muscle.

*Method.* Cats were anaesthetised with ether and luminal of sodium (0.25 grm. per kilo intravenously or intramuscularly) or with luminal of sodium alone. The abdomen was opened from the xiphoid process to within 2 inches of the symphysis pubis. The duodenum was ligated and the abdominal walls retracted in such a fashion that the abdominal cavity formed a bath for the stomach when filled with warm saline.

Records of the contractions were obtained by means of a perforated rubber tube passed into the stomach by the cervical oesophagus and attached to a water float system. A known volume of warm saline was introduced into the stomach, the quantity depending on the tonus condition of the organ.

Other operative procedure in the neck included the introduction of a tracheal cannula, the isolation of the vagi on each side, and, in most cases, the introduction of a cannula into the left carotid for a record of the blood-pressure.

All animals were deprived of food for 24 hours before the experiment. If an active stomach were required, a small meal of meat extract was given an hour and a half before the experiment.

An induction coil as suggested by Martin was used to stimulate the nerves. The secondary coil consisted of 10,000 windings with a resistance of 881 ohms. The coil was calibrated in "Z" units by Martin's method(4). A Lewis adjustable contact breaker was used in the primary circuit to vary the frequency of the stimulations. The intensity was varied by an alteration of the distance between the primary and secondary coils.

*Stimulation of the vagi in the cervical region.* The peripheral ends of both vagi were stimulated together in most cases. If only one were used, the other was sometimes intact and sometimes divided. The results were the same in form in any given stomach condition whether one vagus or both were stimulated, but it was found that stimulation of the left usually caused a greater effect than stimulation of the right, and the greatest effect was caused by stimulation of both together.

*Augmentor Response.* In all experiments, stimulation of the nerves with the stomach in a condition of low tonus caused contraction of the stomach. This effect was the same whether obtained by a single pull, or by slow repeated tapping of the nerve, or again, by currents of high or low frequency and high or low intensity. If the stomach were in an

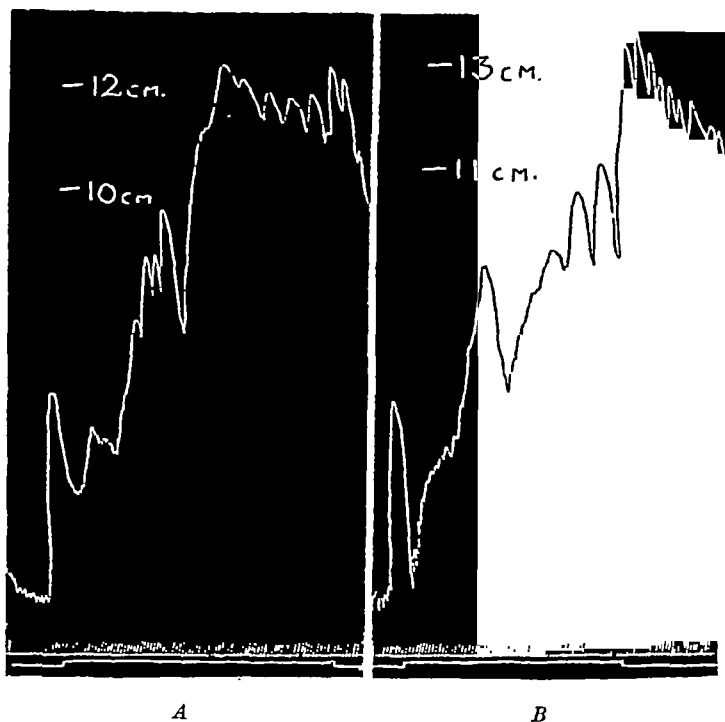


Fig. 1. Both vagi divided; right vagus stimulated. Intensity, 400 "Z" units. Frequency, A, 6; B, 51 per sec. Time tracing, 2 secs. Signal marker displaced 2 mm. to right.

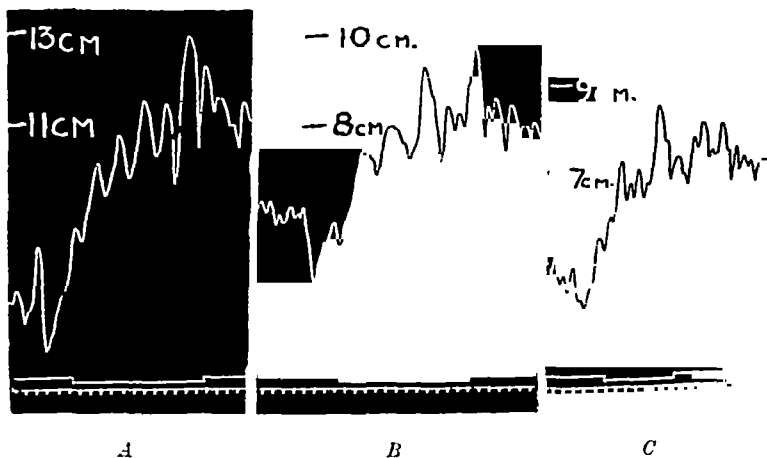


Fig. 2. Both vagi stimulated. Frequency, 51 per sec. Intensity, A, 400 "Z" units; B, 338 "Z" units; C, 250 "Z" units. Time tracing, 10 secs. Signal marker displaced 5.0 mm. to right.

entirely atonic condition, periods of stimulation had to be repeated several times before any effect was observed. When the musculature had some slight degree of tonus, stimulation of the vagus not only caused contraction of the stomach, but also a slight permanent increase in tonus, so that by frequent stimulation the stomach could be raised from a condition of low to one of high tonus. The augmentor response to stimuli of different frequencies is shown in Fig. 1, and to stimuli of different intensities is shown in Fig. 2.

*Inhibitor Response.* In all experiments, stimulation of the nerves with the stomach in a condition of high tonus caused relaxation of the stomach. As before, this response could be obtained by all electrical or mechanical forms of stimulation of the nerve. Relaxation was usually preceded by a slight contraction. In some experiments, the stomach was first in a condition of low tonus, and stimulation of the nerves caused a contraction and a slight permanent rise in tonus. When the stomach tonus had thus become high, stimulation of the vagi with impulses of the same frequency and intensity as had previously caused contraction now caused relaxation. The inhibitor response to stimuli of different frequencies is shown in Fig. 3 and Fig. 4 *A*, and to stimuli of different intensities in Fig. 4, *A*, *B* and *C*.

The sustained contraction obtained in a stomach, after stimulation, is a permanent alteration of its condition. Stimulation of the vagus of an organ with this high tonus always causes relaxation, though the stomach gradually returns to its original condition even though the stimulus be continued. The response of the muscle can be shown by experiment to be determined by the normal condition of tonus and not by the immediately induced condition, for during relaxation if the vagus nerve be again stimulated the organ relaxes further.

*Discussion.* In the experiments described the response of the smooth muscle of the stomach to electrical stimuli depends on the condition of the peripheral mechanism and bears no relation to the frequency or intensity of the stimuli; high tonus predisposing to an inhibitor, low

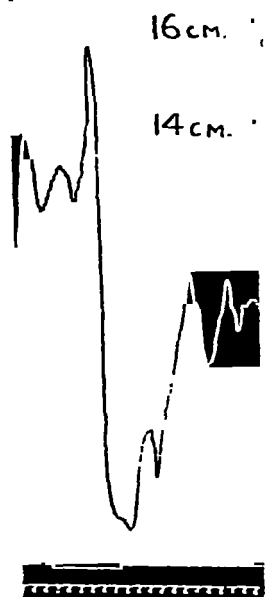


Fig. 3. Both vagi stimulated. Intensity, 400 "Z" units. Frequency, 6 per sec. Time tracing, 10 secs. Signal marker displaced 5 mm. to left. Compare with Fig. 4 *A*.

tonus to a motor response. These observations do not agree with the conclusions put forward by Veach, namely that a current of low

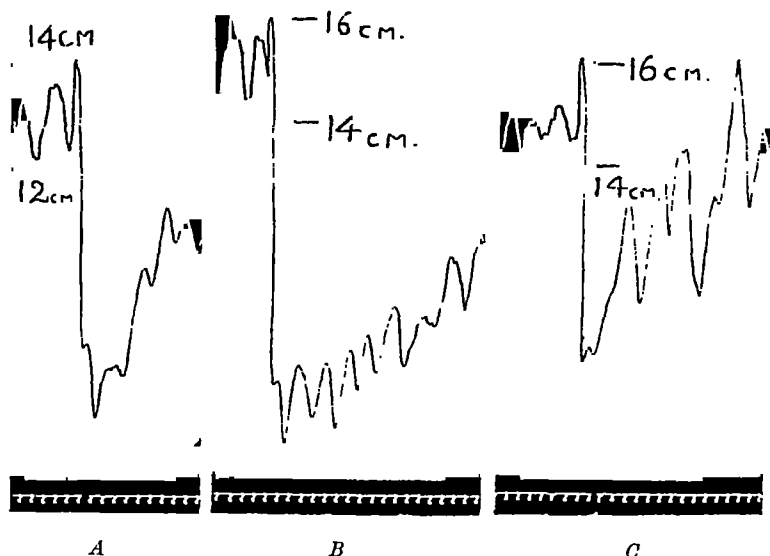


Fig. 4. Both vagi stimulated. Frequency, 51 per sec. Intensity, *A*, 400 "Z" units; *B*, 338 "Z" units; *C*, 250 "Z" units. Time tracing, 10 secs. Signal marker displaced 5.0 mm. to left.

frequency or intensity elicits augmentation, high frequency inhibition of the smooth muscle.

From the experimental results obtained, Veach put forward a theory of the control of contraction and inhibition based on the Wedensky phenomena. It was suggested that the inhibitor response obtained with currents of high frequency was due to an increase of the number of propagated disturbances in the conducting mechanism. On this hypothesis the degree of tonus would be dependent on continuous rhythmic impulses in the peripheral nervous system; increase in rate of conduction with corresponding increase of tonus would account for the greater ease with which inhibition may be produced in preparations possessing high tonus. This mechanism must be limited to impulses arising in the ganglionic plexuses of the muscle wall and not from impulses through the vagus as suggested by Veach, as McCrea, McSwiney and Stopford<sup>(5)</sup> have demonstrated that there is no apparent change in the movements and tone of the stomach on examina-

tion some seven days after uni- or bi-lateral vagotomy or after bi-lateral vagotomy and sympathectomy.

It is possible that Wedensky inhibition might occur in the peripheral system, the rhythm of propagated disturbances being set up in the cells of the ganglion, the addition of further impulses by the vagi bringing about the condition where each impulse travels in the relative refractory period of the last and is therefore reduced to a sub-threshold magnitude. Adrian has shown experimentally that strong stimuli of a given frequency may set up more rapid responses than weak stimuli of the same frequency. The results obtained by us are, however, not in agreement with Veatch's hypothesis, as we have not been able to obtain any relationship between the reaction of the tissue and the frequency or intensity of the stimuli, for with conditions of high tonus, a low frequency or intensity of current, often indeed lower than that used to produce contraction, will produce inhibition: in many experiments a light tap on the nerve fibre will suffice to produce an inhibitor response. Again, initiation of rhythmic movement and rise in tone have been obtained with stimuli of high frequency and intensity. It is difficult therefore to see how the suggested Wedensky phenomena could account for these results.

The observations on stimulation of the sympathetic system made by Veach<sup>(6)</sup> and by McCrea and McSwiney<sup>(7)</sup> do not fit in to the theory. Veach showed that an augmentor or inhibitor response could be obtained, but pointed out that the reaction bore no relation to the frequency or intensity of the stimulus. McCrea and McSwiney have confirmed this and demonstrated the relation between the response of the tissue and the condition of tonus.

McSwiney and Brown<sup>(8)</sup> have shown that a reversal effect can be obtained from smooth muscle using adrenaline. This response is again related to the tonus of the preparation. Recently, in investigations on the response of smooth muscle to alterations of H-ion concentration, it has been possible to reverse the response with adrenaline, the condition of the tissue being altered by variations of the pH. The sustained contraction obtained on increasing the pH cannot be due to impulses in the peripheral nervous mechanism, but must be accounted for by a change in the condition of the tissue proper, as Evans and Underhill<sup>(9)</sup> and more recently McSwiney and Newton<sup>(10)</sup> have shown that the reaction can take place after death of the local nervous system. It is recognised that adrenaline is acting in association with the sympathetic system, and that similar results are not obtained with pilocarpine: yet

it must be emphasised that the tonus in these isolated tissue preparations must be a factor of the tissue and not due to a nervous mechanism.

#### SUMMARY.

Cats were anæsthetised with luminal or with ether and luminal, and records of the movements of the stomach were taken by means of a stomach tube introduced through the cervical œsophagus and attached to a water manometer. The pylorus was ligated. The vagus nerves were isolated and divided in the neck, and the peripheral end stimulated with induction shocks of different frequencies and intensities. Tracings of the stomach movements show the response of that organ to vagus stimulation to depend not upon the frequency or intensity of the stimulus, as suggested by Veach, but upon the condition of the peripheral mechanism. In conditions of low tonus, stimulation of the vagus with low or high frequencies or intensities causes contraction of the stomach and an increase in tonus. In conditions of high tonus, stimulation of the vagus with low or high frequencies or intensities causes relaxation of the stomach.

The expenses of this research have been in part defrayed by the Government Grants Committee of the Royal Society.

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## THE CORONARY CIRCULATION. I. The effect of changes of the blood-pressure and of the output of the heart.

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THE various factors affecting the coronary circulation may be either of a purely vascular origin such as changes in the tone of one or another part of the coronary system or they may be due to changes in the activity of the cardiac muscle, such as changes in the heart rate, in the strength of contraction or in the duration of the various phases of the cycle. The changes in the activity of the heart may, in their turn, affect the coronary circulation directly as an externally applied force compressing the blood vessels or they may act indirectly by producing changes in the aortic blood-pressure, that is, by altering the pressure perfusing the coronary blood vessels. Any of these factors may alter the coronary blood flow or change the distribution of the flow between the various phases of the heart beat.

The effect of blood-pressure on the coronary circulation has been studied by previous observers mainly from the point of view of perfusion pressure, and all authors agree that a rise of the aortic blood-pressure increases the coronary blood flow, and that in this respect the heart does not differ from any other organ. This is confirmed by experiments on the isolated heart(1, 2), on various forms of heart-lung preparations of Starling's(3, 4) and de Barenne's type(5), on the innervated heart-lung preparation(6) and on the whole animal(7). In spite of the large number of experiments and of the general agreement on the main issue it remains far from clear to what extent variations in the coronary blood flow produced by changes of the aortic pressure are due to alteration of the perfusion pressure, and to what extent they are due to changes in the contraction of the heart itself. As regards the second question, Porter(2) expressed the view that "the emptying of the intramural vessels by the contraction of the heart favours the flow of blood through the heart walls chiefly by the diminished resistance which the empty blood vessels offer to the inflow from the aorta when the heart relaxes."



It is claimed that an increase in the strength of contraction increases the coronary blood flow because of a more perfect emptying of the intramuscular vessels by the contraction of the fibres around them. It would follow, therefore, that an increase in the aortic pressure increases the coronary circulation not only on account of a higher perfusion pressure but also on account of the increased strength of contraction of the ventricle. According to this theory of "massaging action" an increased strength of beat as well as an increased heart rate favour the coronary circulation.

In order to study the influence of the arterial pressure independently of the effect it may have as a pressure perfusing the coronary vessels, a special technique had to be devised.

*Method.* The experiments were performed with the ordinary heart-lung preparation on dogs weighing between 10 to 12 kilos. The animals were anæsthetised with morphia and chloralose (0.1 grm. per kilo). After establishing the heart-lung preparation a stretch of about a half centimetre of the circumflex branch of the left coronary artery was dissected close to the aorta, a cannula was placed into the peripheral end of the artery and connected with a reservoir containing blood. The introduction of the cannula into this or any other of the main branches of the coronary arteries presents no difficulty if the precaution is taken to lower the temperature of the heart to about 33° C. At a low temperature the coronary circulation can be interrupted for a minute or two without causing fibrillation; moreover, the slower heart beat makes the introduction easier. In most of our experiments the period of interruption of the circulation in the artery did not exceed 20 seconds. After the establishment of the perfusion the temperature of the heart was raised to 36–37° C. The perfusion of one or other branch of the coronary arteries was made with the same blood as circulated in the heart-lung preparation. Since the blood was defibrinated, it had to be well filtered to free it from all clots. Enough stress cannot be laid on the importance of thorough filtration. We repeated the filtration of the blood through fine linen until it would run through freely and this takes five filtrations or more. Without this precaution the coronary blood flow diminishes progressively throughout the experiment and the preparation lasts for only a short time. In our experiments the blood was filtered in addition immediately before every filling of the reservoir connected with the coronary artery. The blood which was to be used for the perfusion was made to circulate in the heart-lung preparation for about half an hour, a precaution which is important since otherwise defibrinated blood causes

vigorous and protracted vasoconstriction. We cannot confirm the observation of Bornstein(s) that defibrinated blood which is kept for 24 hours loses its vasoconstrictor properties. With these few precautions the preparation remains in a good condition for 5-6 hours.

The general arrangement of the experiment is shown in Fig. 1. It can be seen that the reservoir supplying the artery with blood is connected with a volume recorder and with a hot wire anemometer; the first is used for registration of the blood flow, the second for the determination of the distribution of the flow between the phases of the cycle. The volume recorder was disconnected every time a registration was being made with the hot wire. The registration is based on the cooling of a hot platinum wire in a stream of air. The larger the outflow of blood from the reservoir the greater will be the cooling and, therefore, the greater will be the change in the electrical resistance of the wire, the instrument thus recording the rate of outflow of blood at any moment. The use of the hot wire anemometer for registration of blood flows is described elsewhere(9). We need only mention that since we recorded the flow

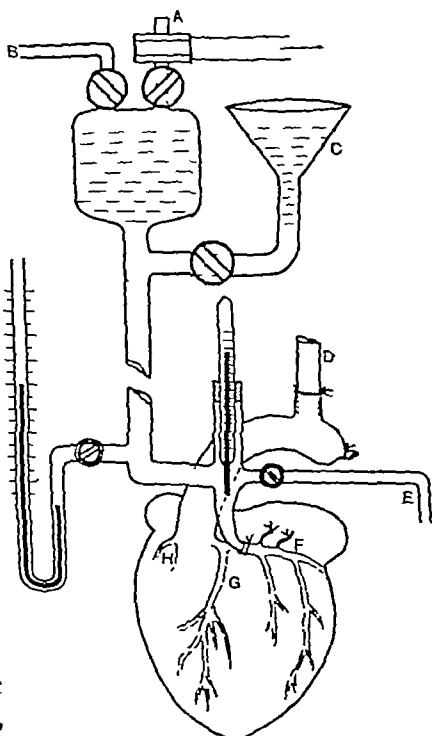


Fig. 1. General arrangement: A, hot wire container with leads to string galvanometer; B, tubes connecting the reservoir with a small spirometer for continuous registration; C, funnel to fill the reservoir with blood; D, cannula leading to the heart-lung apparatus (the venous cannula from the apparatus to the heart is not shown); E, side branch of the coronary arterial cannula, used for measuring back flow; F and G, the circumflex and the descending branches of the left coronary artery; H, the right coronary artery.

through only one of the main branches of the coronary arteries the sensitivity of the anemometer had usually to be somewhat greater than that used for registration of the outflow of blood from the coronary sinus. Lead and glass tubes 5 mm. in diameter were used for all connections. A part of the lead tube was immersed in a water bath in order to warm the coronary blood to any desired temperature. The introduction between the coronary artery and the recording apparatus of

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to zero during the maximal contraction of the ventricle. The flow then increases and soon reaches a steady level. The auricular contraction is

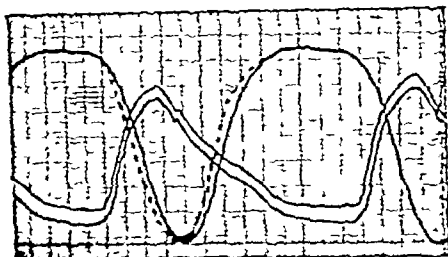


Fig. 2. A typical example of a hot wire record of the inflow of blood into the circumflex branch of the left coronary artery, together with an optical record of the aortic pressure. The dotted line shows the corrected hot wire record. The coronary perfusion pressure and the mean aortic pressure were maintained at 105 mm. Hg. This and the rest of the tracings are to be read from left to right. Time in this and the rest of the tracings in 0.04 seconds unless otherwise mentioned.

imperceptible on the hot wire record. Contrary to the suggestion of Langendorff and Spalteholz, we find no further increase in the coronary flow during the isometric phase of contraction; during this phase the inflow proceeds at a steady level and diminishes only after the contraction of the heart has continued for some time. These time relations depend to a large extent on the relative height of the aortic and coronary pressures. The higher the aortic pressure the nearer the moment at which the coronary flow begins to decline approaches the onset of systole so that with low coronary pressure and high aortic pressure the diminution in the flow may start well within the isometric period. If, on the other hand, the aortic pressure is considerably lower than the coronary perfusion pressure, the effect of systole is delayed so that the coronary flow may continue at a high level during the early part of the ejection phase. These relations are shown in Fig. 3 *a* in which the hot wire records taken at various aortic pressures are superimposed in relation to the beginning of the ejection phase. The higher the aortic pressure the stronger is the contraction of the heart and the sooner does this contraction restrict the coronary inflow. Converse relations are observed as regards the moment of the beginning of the flow in diastole. The higher the aortic pressure relatively to the coronary pressure the more delayed is the moment at which the inflow starts. However, the effect of variations of the aortic blood-pressure on the filling of the blood vessels during diastole is comparatively small, the

a comparatively long tube did not affect the accuracy of the registration. A sudden arrest of the flow produces under these conditions a deflection of the string galvanometer which can be nearly superimposed upon that obtained without the connecting tube. At the sensitivity of the galvanometer and at the temperature of the hot wire used the deflection is in its main part exponential in character and, therefore, is suitable for correction. The correction which has to be applied to allow for the lag of the instrument has been described in a previous paper(9). We found no bad effect from introduction of lead into the perfusion system; it is convenient since it almost entirely eliminates rubber connections which are apt to distort the record. The blood-pressure in the aorta was measured in every experiment immediately above the valves with an optical and a mercury manometer, the latter being disconnected by means of a tap whenever the hot wire and the optical manometer were in use. Most of the experiments were made with perfusion of the circumflex branch of the left coronary artery. This branch supplies the greater part of the external wall of the left ventricle. Injections after the end of the experiment show that the right ventricle and the intraventricular septum are not directly supplied by the circumflex artery, which is corroborated by the anatomical researches of Spalteholz(10). The small branches which leave the artery to supply the auricle were included in the perfusion in some experiments only.

The method just described enables us to study the effect of changes in the blood-pressure, output and heart rate at a constant pressure in the perfused artery and eliminates the direct influence of changes in the aortic pressure on the coronary blood flow.

*The inflow of blood into the coronary artery in relation to the cardiac cycle.* The experiments were usually started with the perfusion pressure kept at the same height as the mean aortic pressure. In confirmation of previous observers(1, 2, 9) we found that under these conditions the coronary blood flow is arrested during systole and is maximal during diastole. Langendorff, who studied the coronary inflow in the isolated heart, reports that during the auricular contraction there is an augmentation of the inflow; we find no such effect. In our experiments the coronary flow was unaffected by the auricular contraction even when the auricular branches of the circumflex artery were perfused together with the ventricular branches. A typical example of a hot wire record taken together with an optical record of the aortic pressure is given in Fig. 2. The diminution of the inflow starts in this case slightly before the beginning of the ejection phase. The flow rapidly declines and falls

pressure the blood vessels are not only filled after the heart has relaxed but they are also expanded. The slow rate of the increase in the inflow at a constant perfusion pressure depends on the viscous properties of the blood vessels. It takes time to stretch them with any given force from any given length to another. To make the stretch quicker a bigger force has to be employed. The vessels have been allowed to contract owing to the contraction of the heart muscle, and when the muscle suddenly relaxes the vessels, being in a contracted state, resist by the viscosity of their walls any sudden attempt to extend them. It is impossible to decide at present to what extent this viscous resistance is due to an active tone and to what extent it is due to the inherent viscosity of the smooth muscle. To find a high tone in the coronary system would not be surprising if one remembers the enormous dilation which it undergoes under the influence of amyl nitrite or anoxæmia.

The gradual decrease in the resistance to the inflow explains why in the majority of our experiments there was no evidence of such an increase in the blood flow immediately following the relaxation of the heart as would suggest "massaging action." At fast heart rates the inflow reaches a maximum just before the next contraction; at slow heart rates the inflow reaches a maximum earlier and then it remains steady for the rest of diastole. We have, however, experiments on record in which the augmentation of the flow during the relaxation of the heart was so rapid that the quickest inflow occurred during the first half of diastole, slightly diminishing during the second half. This "overshoot" of the coronary inflow, which is suggestive of massaging action, is insufficient to counter-balance the restricting effect of systole on the coronary blood flow. It was usually observed only towards the end of an experiment. The condition determining this effect probably depends on the loss of tone of the coronary arteries, at any rate our experiments show that an increase in the strength of contraction alone does not produce it.

As regards the interpretation of the hot wire records it is obvious that under constant conditions the extent of diminution of the flow during systole is determined by the strength of contraction. The level of the inflow during diastole depends on several factors such as the tone of the coronary system, the height of the intraventricular diastolic pressure and to some extent the diminution of the resistance of the coronary blood vessels after they have been emptied by the preceding contraction.

*Effect of changes of the aortic pressure on the coronary blood flow.* Continuous registration with the volume recorder shows that the coronary

onset of the filling being delayed at high pressures only by a few hundredths of a second. The rate of the increase of inflow is not affected to any appreciable extent.

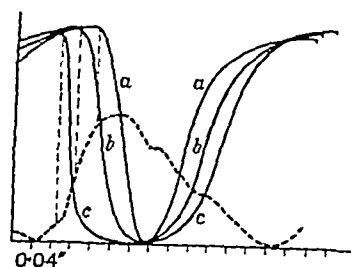


Fig. 3 a

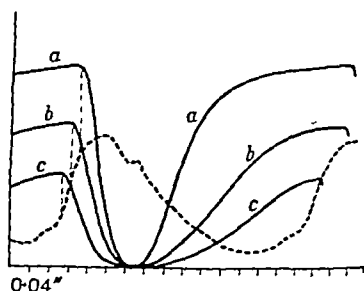


Fig. 3 b

Fig. 3 a. Effect of changes of the aortic pressure on the time relations between the various phases of the coronary circulation and of the cardiac cycle. The perfusion pressure was constant at 100 mm. Hg; the mean aortic pressure in *a*, *b* and *c* was 125, 100 and 86 mm. Hg respectively. The dotted tracing represents the optical record of the aortic pressure at a mean pressure of 100 mm. Hg.

Fig. 3 b. Effect of changes of the perfusion pressure at a constant mean aortic pressure. The aortic pressure was maintained at 100 mm. Hg; the perfusion pressure in *a*, *b* and *c* was 120, 82 and 66 mm. Hg.

The effect of changes of the perfusion pressure on the period of diminution of the inflow during systole is fundamentally the same as that of changes of the aortic pressure. The higher the perfusion pressure (at a constant aortic pressure) the more delayed is the moment at which the diminution of the inflow begins. As regards the filling phase it is considerably more affected by variation of the perfusion pressure than by variations of the aortic pressure. The beginning of the inflow is more conspicuously delayed and the rate of increase of the inflow becomes considerably slower at low perfusion pressures. Fig. 3 b, in which three re-drawn corrected curves of inflow are superimposed in relation to the beginning of the ejection phase, show the extent to which the rate of the increase of flow is affected by changes of the perfusion pressure. It is obvious that the flow proceeds against a considerable resistance which is only gradually removed during diastole. This resistance cannot be due to some residual contraction of the heart muscles since the intra-ventricular pressure shows that relaxation of the muscle is complete very shortly after the dicrotic notch. The only interpretation which seems to explain the slow and gradual increase of the coronary inflow is that the coronary blood vessels themselves constitute this resistance on account of their peripheral tone. Under the influence of the perfusion

pressure the blood vessels are not only filled after the heart has relaxed but they are also expanded. The slow rate of the increase in the inflow at a constant perfusion pressure depends on the viscous properties of the blood vessels. It takes time to stretch them with any given force from any given length to another. To make the stretch quicker a bigger force has to be employed. The vessels have been allowed to contract owing to the contraction of the heart muscle, and when the muscle suddenly relaxes the vessels, being in a contracted state, resist by the viscosity of their walls any sudden attempt to extend them. It is impossible to decide at present to what extent this viscous resistance is due to an active tone and to what extent it is due to the inherent viscosity of the smooth muscle. To find a high tone in the coronary system would not be surprising if one remembers the enormous dilation which it undergoes under the influence of amyl nitrite or anoxæmia.

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*Effect of changes of the aortic pressure on the coronary blood flow.* Continuous registration with the volume recorder shows that the coronary



blood flow is dependent not only on the perfusion pressure but to a large extent also on the aortic pressure. But while an increase in the perfusion pressure increases the flow through the perfused artery, an increase in the aortic pressure diminishes it. The effect of fall or rise in the aortic pressure is very rapid, the coronary blood flow reaching its new level in either case within a few heart beats. The inflow then remains at this new level so long as the relation between the perfusion pressure and the aortic pressure remains constant. Table I gives a few examples of the effect of changes of the aortic pressure on the flow through the perfused artery.

TABLE I.

Exp.	Perfusion pressure mm. Hg	Mean aortic blood-pressure mm. Hg	Blood flow through perfused coronary artery c.c. per min.
1	60	160	11.2
		60	17.2
		30	22.8
2	70	44	30.8
		100	22.6
		148	16.6
		172	5.2
3	115	40	25.0
		60	20.6
		110	16.0
		140	13.5
		40	26.8

Fig. 4 shows two actual records of the coronary inflow. It can be seen how definite and rapid is the change in the flow on increasing or decreasing the aortic pressure.

The greater the increase in the aortic pressure the more pronounced is the diminution of the blood flow through the perfused artery. The effect is more conspicuous towards the end of an experiment, or in hearts which are weak to start with, or which became weakened during the progress of the experiment. In order to bring about a diminution of the blood flow it is not necessary to raise the aortic blood-pressure above that of the coronary perfusion pressure. In fact the effect of changes in the aortic pressure below the level of the perfusion pressure is usually as evident as that of changes in the aortic pressure above that level.

The mechanism of this effect is revealed by the hot wire records taken at different aortic pressures. On comparing the three records of Fig. 5 it becomes evident that the blood flow during diastole remains unaffected by the change in the aortic pressure, and that the diminution of the flow during systole becomes more pronounced at the higher aortic

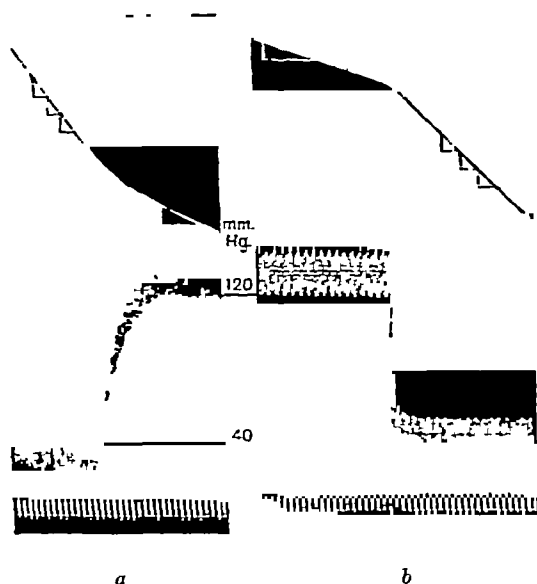


Fig 4. Effect of rise and fall of the aortic blood pressure on the inflow into the left coronary artery. The coronary blood flow was recorded by means of a small spirometer. The step-like lines on the blood flow tracing show the time of inflow of 1 cubic centimetre of blood. The coronary perfusion pressure was in this experiment 95 mm. Hg. The coronary inflow was 30 c.c. per minute at the low aortic pressure and 10.9 and 9.1 c.c. at the high pressure in *a* and *b* respectively. Time in seconds.

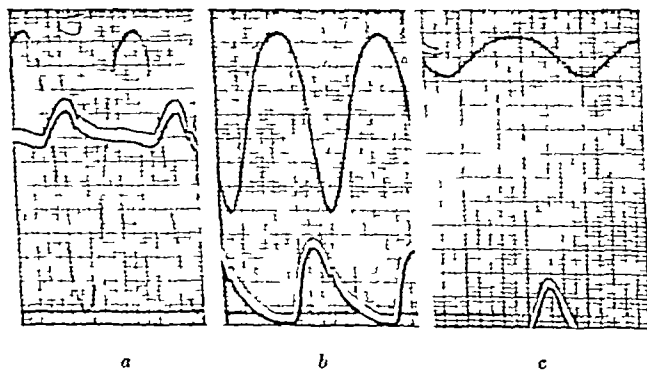


Fig 5. Hot wire records of coronary inflow at mean arterial pressures of 120, 80 and 56 mm. Hg in *a*, *b* and *c* respectively. The perfusion pressure was kept at 100 mm. Hg.

pressures. This increase in the effect of systole is due to the more forcible compression of the blood vessels by the stronger contraction of the heart. In spite of the better emptying of the blood vessels by the strong contraction, the diastolic blood flow in the perfused area remains constant, showing the absence of the massaging effect and of any changes in the tone of the coronary system. It is not only the extent of the diminution of the blood flow during systole which is increased at high aortic pressure but also its duration. At high aortic pressures the systolic diminution of the blood flow begins earlier, it is more profound and it lasts longer.

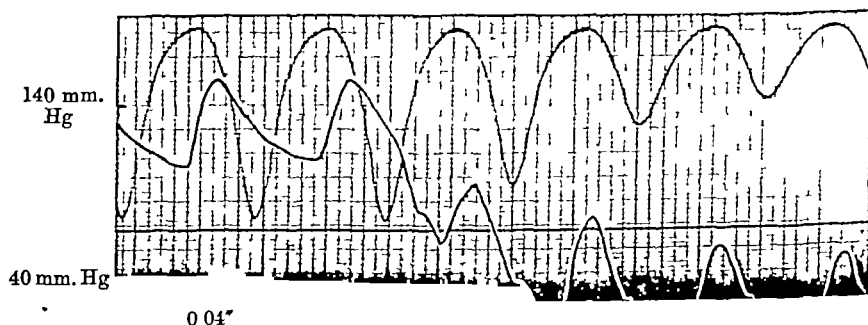


Fig. 6. Effect of a sudden diminution of the aortic blood-pressure on the coronary inflow.

Fig. 6 illustrates the rapidity with which a change in the aortic blood-pressure influences the coronary circulation in the perfused area.

The reason why changes in the aortic blood-pressure have a smaller effect in a strongly beating heart is also made clear by the hot wire records. The strongly beating heart arrests the coronary flow in the perfused artery even when the aortic pressure is considerably below the perfusion pressure, and a rise in the former only slightly prolongs this arrest. In a weak heart beating under the same conditions the coronary flow is not stopped by the systole but only more or less diminished, and the strengthening of the contraction brought about by the increase in the aortic pressure not only prolongs this period of diminution but also intensifies the diminution and finally leads to an arrest of the blood flow during systole. A strong contraction of the heart exerts a pressure upon the coronary blood vessels which must be considerably greater than the aortic pressure, since the blood flow in the perfused area will be stopped by the systole even when the perfusion pressure is considerably higher than the systolic intraventricular pressure. In a weak heart, on the

other hand, if the perfusion pressure is raised to a level slightly higher than, or equal to, the systolic intraventricular pressure the blood flow through the perfused artery continues throughout the cycle and is only more or less diminished during systole.

It is well known that in the heart-lung preparation the coronary circulation gradually increases with the progress of an experiment. Hilton and Eichholtz<sup>(11)</sup> showed that this increase is not due to an accumulation of "metabolites" since replacement of the whole blood in the circulation by fresh blood fails to bring the coronary blood flow to its original level. They refuse to advance any explanation of this spontaneous increase in the coronary circulation. As a result of hot wire registration it is possible to explain the augmentation of the coronary circulation by the gradual diminution in the strength of the contraction of the heart. At the beginning of an experiment the coronary circulation in the perfused artery is completely stopped during a long period of the systole; as the experiment proceeds the arrest of the flow lasts for a shorter time, and if the coronary perfusion pressure is higher than the aortic pressure the blood flow fails to be stopped but is only diminished by the weakened contraction. This progressively smaller effect of the cardiac contraction upon the coronary circulation is observed in every experiment, and it must be regarded with the progressive dilation of the blood vessels as one of the contributory factors which lead to the increase in the coronary circulation in a protracted experiment.

*The back flow effect.* The old view that the coronary arteries are terminal must be regarded on the ground of recent researches as erroneous. In our experiments the collateral circulation between the perfused area and the blood vessels which retain their connection with the aorta could easily be measured. On disconnecting the reservoir perfusing the coronary artery and opening the side branch (*E* in Fig. 1) some blood is seen to flow from the peripheral end of the coronary artery. This blood flows in jerks synchronous with the systoles and the flow is the larger the higher the aortic pressure. For instance, in one experiment at mean aortic pressures of 44, 81.5, 100, 123, 155 and 172 mm. Hg the back flow was 1.8, 3.2, 5.1, 6.0, 6.6 and 7.2 c.c. per minute respectively.

This collateral circulation is entirely sufficient to maintain the activity of the heart; we found in many experiments that an arrest of perfusion by clamping the coronary artery is much more dangerous for the heart than discontinuing the forward perfusion with an open by-path so as to enable the back flow to proceed freely. The extent of the collateral circulation varies greatly from heart to heart, and while in some experiments

pressures. This increase in the effect of systole is due to the more forcible compression of the blood vessels by the stronger contraction of the heart. In spite of the better emptying of the blood vessels by the strong contraction, the diastolic blood flow in the perfused area remains constant, showing the absence of the massaging effect and of any changes in the tone of the coronary system. It is not only the extent of the diminution of the blood flow during systole which is increased at high aortic pressure but also its duration. At high aortic pressures the systolic diminution of the blood flow begins earlier, it is more profound and it lasts longer.

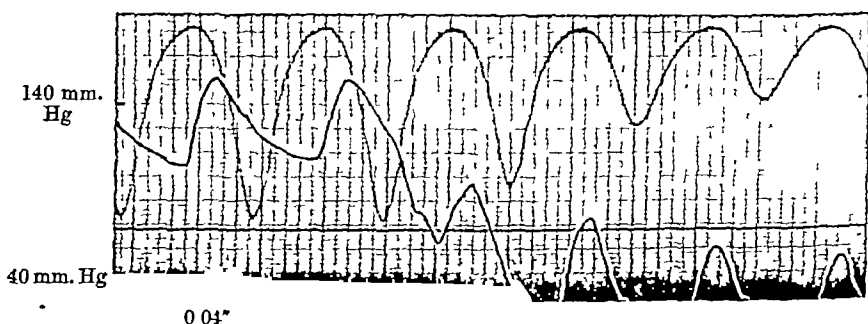


Fig. 6. Effect of a sudden diminution of the aortic blood-pressure on the coronary inflow.

Fig. 6 illustrates the rapidity with which a change in the aortic blood-pressure influences the coronary circulation in the perfused area.

The reason why changes in the aortic blood-pressure have a smaller effect in a strongly beating heart is also made clear by the hot wire records. The strongly beating heart arrests the coronary flow in the perfused artery even when the aortic pressure is considerably below the perfusion pressure, and a rise in the former only slightly prolongs this arrest. In a weak heart beating under the same conditions the coronary flow is not stopped by the systole but only more or less diminished, and the strengthening of the contraction brought about by the increase in the aortic pressure not only prolongs this period of diminution but also intensifies the diminution and finally leads to an arrest of the blood flow during systole. A strong contraction of the heart exerts a pressure upon the coronary blood vessels which must be considerably greater than the aortic pressure, since the blood flow in the perfused area will be stopped by the systole even when the perfusion pressure is considerably higher than the systolic intraventricular pressure. In a weak heart, on the

other hand, if the perfusion pressure is raised to a level slightly higher than, or equal to, the systolic intraventricular pressure the blood flow through the perfused artery continues throughout the cycle and is only more or less diminished during systole.

It is well known that in the heart-lung preparation the coronary circulation gradually increases with the progress of an experiment. Hilton and Eichholtz(11) showed that this increase is not due to an accumulation of "metabolites" since replacement of the whole blood in the circulation by fresh blood fails to bring the coronary blood flow to its original level. They refuse to advance any explanation of this spontaneous increase in the coronary circulation. As a result of hot wire registration it is possible to explain the augmentation of the coronary circulation by the gradual diminution in the strength of the contraction of the heart. At the beginning of an experiment the coronary circulation in the perfused artery is completely stopped during a long period of the systole; as the experiment proceeds the arrest of the flow lasts for a shorter time, and if the coronary perfusion pressure is higher than the aortic pressure the blood flow fails to be stopped but is only diminished by the weakened contraction. This progressively smaller effect of the cardiac contraction upon the coronary circulation is observed in every experiment, and it must be regarded with the progressive dilation of the blood vessels as one of the contributory factors which lead to the increase in the coronary circulation in a protracted experiment.

*The back flow effect.* The old view that the coronary arteries are terminal must be regarded on the ground of recent researches as erroneous. In our experiments the collateral circulation between the perfused area and the blood vessels which retain their connection with the aorta could easily be measured. On disconnecting the reservoir perfusing the coronary artery and opening the side branch (*E* in Fig. 1) some blood is seen to flow from the peripheral end of the coronary artery. This blood flows in jerks synchronous with the systoles and the flow is the larger the higher the aortic pressure. For instance, in one experiment at mean aortic pressures of 44, 81.5, 100, 123, 155 and 172 mm. Hg the back flow was 1.8, 3.2, 5.1, 6.0, 6.6 and 7.2 c.c. per minute respectively.

This collateral circulation is entirely sufficient to maintain the activity of the heart; we found in many experiments that an arrest of perfusion by clamping the coronary artery is much more dangerous for the heart than discontinuing the forward perfusion with an open by-path so as to enable the back flow to proceed freely. The extent of the collateral circulation varies greatly from heart to heart, and while in some experiments

it was as large as in the example given above, in others it was barely a quarter of that amount. Provided the aortic pressure is not changed the back flow remains constant during long periods of observation, showing that it is not due to an emptying of the perfused area of blood which filled it during the preceding period of perfusion but a true collateral circulation.

In the above experiment the back flow was measured with the peripheral end of the coronary artery open to the air. Under these conditions the contraction of the heart expels the blood from the coronary system in a forward direction through the coronary veins and in a backward direction through the open artery. When the artery is connected with the reservoir and is under pressure the contraction of the heart expels the blood only in the forward direction through the veins. However, if the aortic pressure is considerably higher than the perfusion pressure, and if the perfused area is filled with blood either on account of vaso-dilation or on account of large collateral connections, then the strong contraction of the heart may raise the pressure in the coronary blood vessels above the perfusion pressure and a back thrust of blood may be initiated. The higher the aortic pressure above the perfusion pressure the greater is the back thrust which finally may become equal to the forward flow occurring during diastole or even exceed it. Under these conditions the net coronary circulation, as recorded by the continuous method of registration, is zero or even changes into a flow in the reverse direction. Records exemplifying this case are given in Fig. 7.

In Fig. 7, *e* the blood flow as revealed by the tracings is nearly arrested and in Fig. 7, *f* there was an unmistakable back flow. The hot wire registration makes the mechanism underlying these events clear. The first sign of a back thrust is revealed by a small bifurcation of the hot wire record, a second deflection being observed late in systole at the moment of the maximal contraction of the heart. This deflection grows in

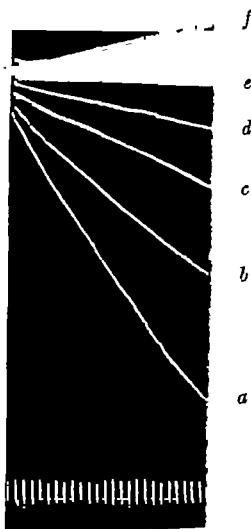


Fig. 7. Inflow into the perfused coronary artery recorded by the continuous method at mean aortic blood-pressures of 40, 58, 70, 100, 120 and 145 mm. Hg from *a* to *f*. The perfusion pressure was kept constant at 55 mm. Hg. Time in seconds. The blood flow from *a* to *f* was 27.3, 15.0, 10.0, 4.6, 1.7 and -7.2 c.c. per minute.

area as the back thrust increases; finally it becomes equal to the forward flow and then it exceeds it. On inspecting Fig. 7, *e* it would seem that the coronary artery is not supplied with blood; actually, however, very violent events occur in the perfused area. Blood enters the system during diastole and fills it by an amount which is determined by the perfusion pressure. The strong contraction of the heart compresses the blood vessels and expels some blood into the veins and some back into the arteries against the perfusion pressure. At the same time some blood is driven from the other coronary blood vessels through the collateral branches into the perfused area so that blood of an aortic origin is thrust into the perfusion reservoir. Hot wire records corresponding to the various conditions of Fig. 7 are given in Fig. 8.

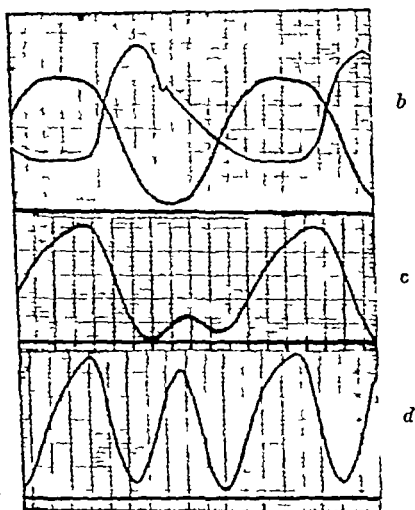


Fig 8 Hot wire records corresponding to conditions *b*, *c* and *d* of Fig. 7.

The blood flow in either direction is recorded in the hot wire method by a deflection of the galvanometer in the same direction. A separation of the back flow from the forward flow by means of valves is incompatible with accurate registration on account of the necessary movement of the valves which, however small, produces considerable distortions of the hot wire record. Attempts at separating the two directions of the blood flow were made; the results could not, however, be treated quantitatively. If under the condition in which, on account of high aortic pressure, the net coronary blood flow

TABLE II.

Coronary perfusion pressure 40 mm. Hg.

Remarks	Mean aortic pressure in mm. Hg	Blood flow through perfused coronary artery in c.c. per minute
Without valve	40	15.8
"	120	9.3
"	150	1.8
With valve	150	5.2
Coronary perfusion pressure raised to 120 mm. Hg.		
With valve	150	25.6
Without valve	150	26.1



is zero (as in Fig. 7, *e*) a valve is introduced into the perfusion system so as to prevent a back flow, a forward flow is immediately initiated. This is observed even with valves which introduce a small resistance to the perfusion (Table II).

The last two readings of the coronary flow in the above table show that the resistance introduced by valves was negligible, the blood flow changing by only a fraction of a c.c. When, however, the valve was introduced in the case of a low coronary pressure at which the hot wire record showed the presence of a considerable back thrust, the blood flow increased from 1.8 to 5.2 c.c. The introduction of the valve greatly reduces the systolic back thrust, as recorded by the hot wire, but does not abolish it altogether on account of the movement of the valve itself (Fig. 9).

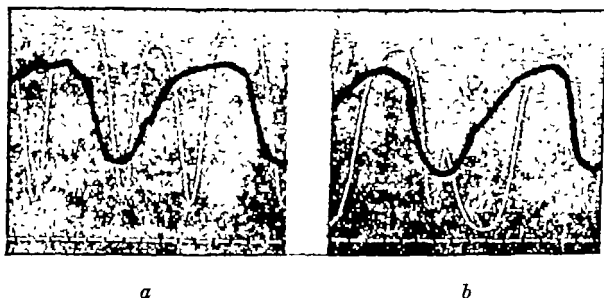


Fig. 9. Effect of introduction of a valve into the perfusion systems. *a* before and *b* after introduction of the valve. The back thrust of blood is conspicuously diminished. The record of the aortic blood-pressure is inverted. The hot wire record is not corrected and the base line in *a* is shifted downwards by 5 mm.

In presence of a valve no real back flow is possible and, therefore, the circulation in the perfused area cannot be arrested or reversed by a high aortic pressure; the blood flow is now diminished only on account of the increased strength of contraction.

A real back flow is observed only when the perfusion pressure is much lower than the aortic pressure. This is probably due to the considerable resistance of the collateral interconnections. The pressure in the peripheral end of the coronary artery through which the perfusion has been stopped is always considerably lower than the aortic pressure (Table III).

The great resistance of the collateral branches explains why the back flow is comparatively small and why it can be observed only when the aortic pressure is very much higher than the perfusion pressure.

TABLE III.

Mean aortic pressure in mm. Hg	Back pressure in the coronary artery in mm. Hg	Back flow through open coronary artery in c.c. per minute
30	6.5	0.3
63	12.5	0.9
115	20.0	1.8
160	26.0	3.9

No back flow is observed during diastole, it is the contraction of the heart which, on getting stronger with a rise of the aortic pressure, raises the pressure in the rest of the coronary system so high that some blood is pressed into the perfused area.

The back thrust of blood which may be observed even in the isolated perfused heart (so far unpublished experiments of Dr Rössler and Dr Pascual) is of the same origin as the true back flow just described. In the first case it is the perfused area which is so strongly compressed by the heart that some blood is thrown back into the arteries; in the second case blood of aortic origin is pressed into the perfused area which is at a lower pressure than that in the aorta.

*Effect of changes of the cardiac output.* The effect of changes of the cardiac output on the coronary circulation in the intact heart has been analysed elsewhere<sup>(12)</sup>; it was found that even considerable changes of the output have no effect on the blood flow from the coronary sinus provided the true mean aortic pressure is maintained constant. In the case of the perfused artery the effect of increasing the cardiac output is fundamentally the same as that of increasing the aortic pressure except that it is smaller. The coronary blood flow through the perfused area is diminished at large outputs on account of the stronger contraction of the heart and therefore a more conspicuous diminution of the blood flow is observed during systole. The blood flow during diastole is not affected by changes in the cardiac output. Table IV serves to illustrate the magnitude of the effect.

TABLE IV.

The coronary perfusion pressure is maintained at 84 mm. Hg.

Mean aortic pressure in mm. Hg	Systemic output in c.c. per minute	Blood flow through the perfused artery in c.c. per minute
80	250	33.4
80	500	31.4
80	1400	25.5
80	500	31.5
106	500	24.5

The nearly threefold increase of the systemic output from 500 to 1400 c.c. per minute had approximately the same effect on the coronary blood flow as an increase of the aortic blood-pressure by 26 mm. Hg. In this experiment the effect of increased output was, if anything, more obvious than usually. A back thrust of blood into the perfusion system can sometimes be observed when the output is increased; this effect is, however, smaller and less constant than in the case of high aortic pressure.

#### SUMMARY AND CONCLUSIONS.

The experiments described in this communication show that the contraction of the heart has no other effect upon the coronary circulation than that of restricting it to a greater or smaller extent. The increase in strength of the contraction which follows a rise of the aortic pressure or an increase in the cardiac output leads to diminution of the coronary blood flow through the perfused artery. The analysis of this effect by the hot wire method shows that it is only during systole that the blood flow is diminished; the flow during diastole remains constant.

1. The blood flow through a perfused branch of the left coronary artery was measured by means of a continuous method of registration and by the hot wire anemometer.

2. The time relations between the various phases of the cardiac cycle and of the coronary blood flow were determined.

3. The higher the aortic blood-pressure the smaller is the blood flow through the perfused artery. This effect is due to (a) the increased strength of contraction of the heart causing a more prolonged and more pronounced diminution of the coronary blood flow during systole, and (b) to a regurgitation of blood by the strongly beating heart into the arterial system.

4. The collateral circulation between different branches of the left coronary artery has been measured. The collateral blood flow can be observed only when the difference in pressure in the two main branches of the left coronary artery is considerable.

5. The effect of increased output is fundamentally the same as that of increased aortic pressure but smaller. At a constant coronary pressure the coronary blood flow through the left artery is the smaller the stronger the contraction of the heart.

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# THE DISTRIBUTION OF PHOSPHORUS COMPOUNDS IN THE BLOOD OF CERTAIN MAMMALS.

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THE objects of this paper are (a) to present analytical figures indicating the variation, from one species of animal to another, in certain types of phosphorus compound in the blood, and (b) to call attention to points of interest arising directly from a consideration of these figures.

Since Abderhalden's<sup>(1)</sup> work in 1897 and 1898 comparative analyses of the blood of different mammals do not appear to have been published. Theories as to possible correlation between the properties of the red cell and its phosphorus content have in the meanwhile been based on his figures for corpuscular phosphorus. When these analyses were made, however, the labile equilibrium existing in blood between the organic phosphorus of the red cells and the inorganic phosphate of the plasma was not realised. In fact it is not even yet generally appreciated that in human blood, for example, the inorganic phosphate present in the blood may rise as much as six-fold if the blood be kept for several hours at 20° or above, and that this increase takes place even more speedily if the blood be laked. Presumably the interrelated variables, hydrogen-ion concentration (particularly within the cells) and buffering power of the blood, the oxygen and CO<sub>2</sub> capacity, and the Donnan equilibrium at the red cell boundary will be affected to a greater or lesser extent when the intracorporal organic phosphorus compounds are hydrolysed. The agent responsible for this hydrolysis is a phosphatase which is mainly concentrated in the cells, and whose activity is increased by hæmolytic<sup>(2)</sup>. Unhæmolytic blood maintains for a short time its inorganic phosphate value unchanged. If there has been much CO<sub>2</sub> loss during drawing and manipulation of the sample, there may even be a temporary decrease in the inorganic phosphate owing to enzymic synthesis of phosphoric esters. It has been shown by Martland<sup>(3)</sup> that in freshly drawn blood there is a delicately balanced equilibrium, controlled by the C<sub>H</sub>, between hydrolysis and synthesis. If the blood is made acid

to pH 7.3 there is hydrolysis, if alkaline to pH 7.35 there is, for a short time, synthesis of phosphoric esters. This, however, soon gives place to hydrolysis and the consequent increase in the inorganic phosphate just mentioned. Precipitation of the blood proteins completely inhibits this process. It is evident therefore that in the determination of the distribution of phosphorus in various types of combination in blood, the proteins should be precipitated with the minimum of delay.

With these precautions in mind, the phosphorus partition in the blood of animals belonging to several different species has been determined. It has been found that whilst there are very wide variations from one species to another, the quantity of acid soluble ester phosphorus per unit volume of red cells is remarkably constant in health from one individual to another within a species. This value has been called, for convenience,

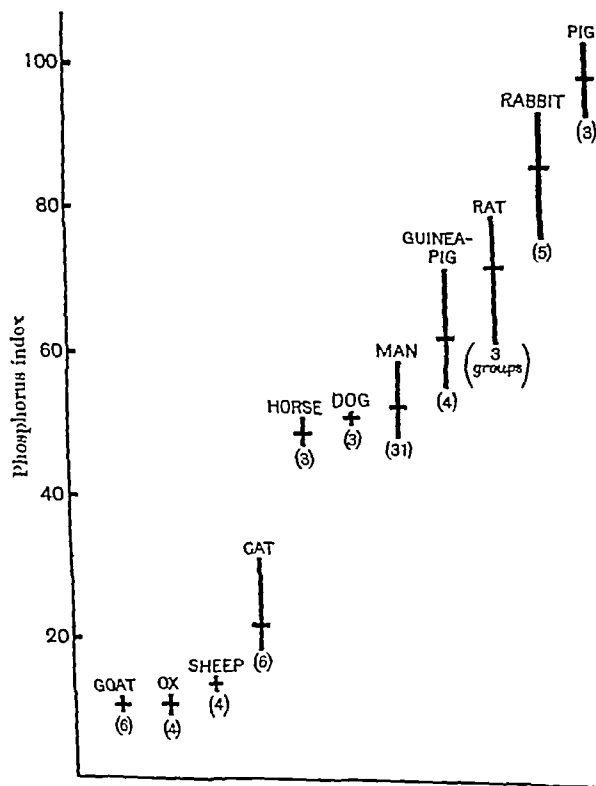


Fig. 1. Species ranged in order of increasing phosphorus index. Vertical line indicates magnitude of variation in the species. Horizontal line indicates mean value. Numbers in brackets indicate number of individuals examined.

TABLE I.

		No. of individuals		mgrm. P			
Species				Free P per 100 c.c. blood	Ester P of red corpuscles per 100 c.c. corpuscles	Enzyme hydrolysable ester P per 100 c.c. corpuscles	Non-hydrolysable ester P per 100 c.c. corpuscles
Pig ...	...	3	Highest	8.5	104	—	—
			Lowest	7.2	93	—	—
			Mean	8.0	97	26	71
Rabbit ...	...	5	H	3.8	94	—	—
			L	2.7	76	—	—
			M	3.3	86	24	62
Rat ...	...	3 groups	H	4.8	79	—	—
			L	4.2	62	—	—
			M	4.5	73	19	54
Guinea-pig		4	H	5.7	73	—	—
			L	2.6	56	—	—
			M	4.4	63	14.5	48.5
*Man ...	...	31	H	3.8	59	—	—
			L	2.4	48	—	—
			M	3.1	53	16	37
Dog...	...	3	H	4.6	52	—	—
			L	2.1	50	—	—
			M	3.1	51	10	41
Horse ...	...	3	H	2.5	51	—	—
			L	2.0	47	—	—
			M	2.3	49	7	42
Cat ...	...	6	H	6.9	31	—	—
			L	4.6	18	—	—
			M	5.6	22	12.2	9.4
*Sheep ...	...	4	H	7.1	14.6	—	—
			L	5.7	12.5	—	—
			M	6.4	13.5	9.2	4.3
*Ox ...	...	4	H	7.2	12.0	—	—
			L	4.9	9.2	—	—
			M	5.8	10.5	6.4	4.1
*Goat ...	...	6	H	6.1	11.7	—	—
			L	4.0	9.5	—	—
			M	5.7	10.3	8.5	1.8

\* (1) Some of these figures have been recorded previously (4), (5).

(2) In no cases were more than traces of phosphoric esters found in the plasmata.

(3) Probably the main source of error in this table lies in the determination of the percentage vol. of red cells by the hæmatocrit. The same type of power driven machine has been used throughout. Duplicates agreed to 1 p.c. or less. To avoid the osmotic effects of too great a concentration of oxalate, heparin has been used as anticoagulant in the small sample of blood required for the hæmatocrit determination.

(4) The "hydrolysable" phosphoric esters comprise adenine nucleotide and probably a hexose phosphate also. The amount of adenine nucleotide in human blood (6) will account for between  $\frac{1}{4}$  and  $\frac{1}{2}$  of the hydrolysable P. The "non-hydrolysable" phosphoric esters probably consist mainly of diphospho-l-glyceric acid (7).

the "phosphorus index" (4). The variations in this index from species to species are such that it is possible by a determination of this one value in a blood taken from one of the animals of our series, but of otherwise unknown origin, to make a fairly shrewd guess as to the species from which the blood was derived.

The methods used for the determination of the P partition in blood have been described (4). The analyses are summarised in Table I, the species being shown in descending order of phosphorus index; the alteration of phosphorus index from one species to another is shown graphically in Fig. 1.

*Correlation of the phosphorus index variation with other variations from species to species.*

It must be acknowledged that there is at present no satisfactory explanation of the functions of these compounds within the highly specialised red cells, and still less any reason for their gross variation from one animal species to another. There are, however, certain suggestive facts and possible correlations brought together here which must be taken into account in endeavouring to ascertain the function of these compounds.

(a) *Diffusibility.* The phosphoric esters appear to be part of the structure of the cells in the sense that until the cells are hæmolysed, the esters as such do not emerge from them. Nevertheless after enzymic autolysis of the esters, i.e. by keeping oxalated blood overnight at room temperature, some of the inorganic phosphate produced may diffuse into the plasma. It is evident that the non-diffusible phosphoric esters, which have the base binding capacity of a dibasic acid for each atom of P in the molecule, must play an important part in the maintenance of the Donnan equilibrium at the cell boundary. It is probable that the red cell membrane is permeable to the inorganic phosphate ion.

(b) *Osmotic effects.* Naturally occurring phosphoric esters in which one hydroxyl group of the acid is esterified act as dibasic acids and dissociate in dilute solution into three ions. On this basis, and assuming that the red cell contains 65 p.c. water available for solvent purposes the osmotic pressure exerted by the non-diffusible phosphoric esters and the base held by them varies from 5 p.c. of the total osmotic pressure of the cell contents in the case of goat's blood to as much as 45 p.c. in the case of pig's blood.

(c) *Size of cells.* On the whole the individual red cells of a blood containing greater quantities of phosphoric esters per 100 c.c. cells (e.g.



pig) are larger than the red cells of a blood containing smaller quantities of esters (e.g. goat). This has also been observed in disease in man, Byrom and Kay<sup>(8)</sup> having shown that the large cells in the blood in pernicious anæmia contain considerably more phosphoric esters per unit volume of cells than normal human erythrocytes.

(d) *Phosphorus content of cells and ease of hæmolysis.* Ryvosh<sup>(9)</sup>, while investigating the comparative resistance of red cells towards a number of hæmolytic reagents, found that the cells of different mammals could readily be ranged in order of resistance to hypotonic solutions of sodium chloride, and that this order was the reverse of that of the resistance to saponin. Port<sup>(10)</sup> supported these findings and endeavoured to correlate the resistance to hæmolysis with the chemical constitution of the cells as given by Abderhalden. He concluded that it was the amount of phosphoric acid in the cell which determined this resistance. A cell with a high phosphoric acid content was less resistant to saponin than a cell containing less phosphoric acid. On the other hand, Ponder<sup>(11)</sup>, who has recently re-investigated the relative resistance to hæmolysis of red cells of a number of mammals using a method which permits of a roughly quantitative estimation of this resistance, has come to the conclusion that there is no real correlation between the phosphoric acid content of the cell and its resistance to saponin although he finds a rank correlation coefficient as high as  $-0.89$  between the "nuclein" phosphoric acid of the red cell (as given in the old figures of Abderhalden) and the resistance to saponin. He believes that the non-hæmoglobin protein content of the cell is the primary factor in the determination of the resistance to saponin and to hypotonic salt solution, and that the observation of Port is due to the fact that there exists a coefficient of rank correlation between protein and phosphoric acid content of  $-0.75$ .

It is not clear from the published figures of any of these authors how many animals of each species have been studied, and no report is made as to the extent of individual variations within the same species. The variation found in the order, as shown by different authors, of the resistance of the erythrocytes both to dilute salt solutions and to saponin may possibly find an explanation in such individual variations. The methods of determining resistance to hæmolysis are, in any case, less exact than those for determining the corpuscular phosphorus content. Ryvosh found, for example, that simple washing of the corpuscles with isotonic salt solution changed the order of resistance, which process certainly does not affect the phosphoric ester content of the corpuscles.

In Table II are summarised the lists published by several authors for

the order of resistance of various red cells to saponin, together with the order of size of the phosphorus quotients, etc., as determined by the present author. [Compare, particularly, columns (d) and (e).]

\*TABLE II.

Resistance against saponin. Most resistance at head of table				Amount of phosphoric esters present. Least at head of table		
(a)	(b)	(c)	(d)	(e)	(f)	(g)
				P quotient	Enzyme hydro- lysable esters	Non- hydro- lysable esters
Meyer(12)	Ryvosh	Port	Ponder	Goat	Ox	(1) { Goat
Ox	Sheep	Sheep	Sheep	Ox	Horse	{ Ox
Sheep	Goat	Ox	Ox	Sheep	Goat	{ Sheep
Dog	Ox	Pig	Goat	Cat	Sheep	{ Cat
Pig	Cat	Dog	Cat	Horse	Dog	(2) { Man
Rabbit	Pig	Man	Pig	Dog	Cat	{ Dog
—	Dog	Rabbit	Dog	Man	Guinea-pig	{ Horse
—	White Rat	—	Guinea-pig	Guinea-pig	Man	{ Guinea-
—	Rabbit	—	Man	—	—	{ pig
—	Guinea-pig	—	Rat	Rat	Rat	(3) { Rat
—	—	—	Rabbit	Rabbit	Rabbit	{ Rabbit
—	—	—	—	—	Pig	{ Pig
				*[Man] Pig		

(1) Low group.

(2) Intermediate group.

(3) High group.

\* Pernicious anæmia.

(1) The pig is the main anomaly, but if we exclude this animal and remember that quantitatively there is little difference in phosphorus quotient between the ruminants, the resemblance in order between ease of saponin hæmolytic and corpuscular content of phosphoric esters is striking, and cannot be due entirely to chance.

(2) In five cases of pernicious anæmia and in three cases of hæmolytic jaundice the corpuscular ester phosphorus was much above the normal.

(3) The grouping in column (g) into low, intermediate and high figures may also be correlated with the grouping given by Abderhalden(13) for the sodium and potassium content of the red cell. In the high group, at least, the base united with the phosphorus esters of the red cells would appear to be potassium.

### Possible correlation with urinary phosphate excretion.

It has been suggested to me by Dr H. E. Magee that the path of elimination of phosphorus from the body may possibly be correlated with the amount of P esters in the red cells. Thus the goat, the ox and the sheep, on a normal diet, excrete practically no phosphate in the urine, whilst man excretes some 50-60 p.c. of his intake, and the dog and the pig also excrete relatively large amounts by the kidney. Much work has been done (*vide* Forbes and Keith(14)) on the effect of varying the diet on the path of excretion of phosphorus from the body, and it is clear that, in every animal investigated, the distribution of phosphorus between the urine and the fæces is controlled to a large extent by the

reaction of the ash of the diet. There is evidence, however, that another factor is also concerned in determining the path of excretion. Unfortunately I have not been able to find in the literature any record of experiment in which the comparative mineral metabolism of a series of animals of varying species, *while living on the same diet*, has been studied. It should be possible to do this, for example, with the goat, the horse, the guinea-pig and the rabbit, animals sufficiently different in their phosphorus index, but which are able to live on the same diet. At present the further discussion of this possible correlation is prevented by lack of such evidence.

#### SUMMARY.

1. Using precautions to avoid the rapid changes which are now known to occur in the phosphorus compounds in shed blood, the phosphorus partition in the blood of animals belonging to several mammalian species has been determined.

2. The quantity of acid-soluble ester phosphorus per unit volume of red cells ("phosphorus index") is remarkably constant in health from one individual to another within a species, but varies markedly from one species to another.

3. In the light of the figures obtained, possible correlations with differences in size of cell, in ease of hæmolysis and in path of phosphate excretion from one species to another are shortly discussed.

This work has been carried out with the aid of a grant from the Research Fund of the London Hospital Medical College.

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# THE INFLUENCE OF ALCOHOL ON ABSORPTION OF GLUCOSE. Part I.

BY NORA EDKINS.

*(From the Physiological Department, Bedford College, London.)*

It is generally stated in modern text-books of Physiology that the stomach has no power of absorbing either water or dissolved substances with the exception of a few compounds such as alcohol, chloral hydrate and strychnine.

Examination of older literature shows that Tappeiner<sup>(1)</sup> (1880), investigating the absorptive power of the stomach in cats and dogs, concluded that glucose and peptone were both absorbed slightly from water solution and definitely absorbed to a greater extent from dilute alcoholic solutions.

Anrep<sup>(2)</sup> and later Segall<sup>(3)</sup> working with dogs with gastric fistulæ concluded that the stomach absorbed both glucose and peptone.

v. Mering<sup>(4)</sup> and simultaneously Brandl<sup>(5)</sup> studied absorption from the stomach also in dogs with gastric fistulæ. v. Mering came to the same conclusion as Edkins<sup>(6)</sup> that water was not absorbed: with regard to dissolved substances he found considerable absorption of glucose and peptone from water solutions and an increased absorption of glucose in the presence of alcohol. Brandl, whose experiments were all performed on one dog, records very high figures for the absorption of glucose, peptone and sodium iodide with a definite increase in the presence of alcohol. His work is open to adverse criticism since he found absorption of water from quite concentrated solutions which was denied by v. Mering. Other workers, as Ryan<sup>(7)</sup> and Brequet<sup>(8)</sup>, state that alcohol does not enhance the absorption of strychnine by the stomach and according to Tchekounov<sup>(9)</sup> saccharose is not absorbed in the slightest degree from water or alcoholic solutions in the stomach.

Most experiments carried out on the effect of alcohol on intestinal absorption, with the exception of those of Nakamura<sup>(11)</sup> have been done on dogs with Thiry-Vella fistulæ. It must be urged against this method that there always results a catarrhal condition of the gut and

therefore it cannot constitute a reliable method for studying physiological absorption (see the discussion in Schäfer's *Textbook of Physiology*, 1898, I, p. 557). Scanzoni<sup>(10)</sup> employed T.-V. fistulæ and found that various drugs which acted as stimulants (including alcohol) had a much smaller effect on intestinal absorption than on gastric absorption. Nakamura's<sup>(11)</sup> conclusion, working with the "acute" experiment in animals anæsthetised by A.C.E. mixture, was that alcohol does not promote intestinal absorption.

The above brief review of past work on absorption by the stomach and the effect of alcohol on absorption from the alimentary canal generally shows that no definite statements can be made.

Using decerebrate animals in the absence of anæsthetics (ether was used in the operation of decerebration) N. Edkins and M. M. Murray<sup>(12)</sup> found that alcohol accelerated the absorption of CO<sub>2</sub> in the stomach. Experiments were then undertaken with the same operative procedure to see if alcohol accelerated absorption generally.

In the present investigation attention was first directed to the disappearance of glucose alone from the stomach cavity and then to the effect that alcohol exhibited on the rate of disappearance.

#### METHOD.

Cats were anæsthetised with ether, the carotids ligatured and decerebration performed by trephining. The respiratory centre functioned spontaneously. The animals were immersed in a saline bath at 37° C. The stomach was exposed, the œsophagus ligatured and a tube tied into the pylorus. Great care was taken to maintain the circulation intact, the appearance of the blood vessels readily informing on this point. The stomach was thoroughly washed out with warm saline; 25 c.c. of glucose solution with or without alcohol was introduced, and the glucose remaining in the introducing tube was washed into the stomach. Every experiment consisted of two parts, each part lasting for an hour, in one period glucose alone was given, in the other both sugar and alcohol. The actual concentration of the sugar was practically identical in all cases, viz. 20 p.c. The strength of the alcohol was 10 p.c., in most cases the actual amount of alcohol present being 2.5 c.c. In some of the later experiments this strength was doubled. At the end of the hour the contents were withdrawn into a 200 c.c. graduated flask and the stomach washed out with water until the volume was approximately 200 c.c. The order of the administration of glucose alone and glucose with alcohol was alternated in successive experiments. The estimations of glucose

in the solutions introduced and removed were made by Bertrand's method. All solutions removed from the stomach were freed from any protein by the Folin-Wu technique. Any loss that might have occurred in the process of introduction and removal by adsorption or mechanical adhesion to the gastric mucous membrane was estimated by introducing a known volume of solutions and immediately removing the same, and such determinations showed that any error in the results from this cause would not exceed 0.05 gram.

## RESULTS.

Periods of one hour; 25 c.c. of 20 p.c. glucose introduced either in water or in 10 p.c. alcohol. Total glucose 5 gram.

## Disappearance of glucose from stomach.

A.	Exp.	1st hour glucose with	2nd hour glucose
		alcohol (gram.)	alone (gram.)
	I	0.881	0.46
	II	0.625	0.0
	III	0.475	0.0
	IV	0.72	0.58
	Mean	0.68	0.26

B.	Exp.	1st hour glucose	2nd hour glucose with
		alone (gram.)	alcohol (gram.)
	V	0.41	1.006
	VI	0.31	0.31
	VII	0.28	0.58
	VIII	0.38	0.44
	IX	0.28	0.60
	X	0.28	1.06
	Mean	0.32	0.67

From the foregoing table it can be seen that glucose disappeared from the liquid in the stomach to the extent of approximately 0.3 gram. per hour. Also that with one exception the disappearance of glucose when given with alcohol was greater irrespective of whether the glucose alone was given in the first or second period. In the presence of alcohol the absorption of glucose was 0.67 per hour, that is, the disappearance with alcohol was at twice the rate that it was without.

Other experiments to test the rate of absorption of glucose with or without alcohol in the intestine showed that the influence of alcohol was here less marked. In these experiments about 100 cm. length of small intestine was taken distal from the duodenum. Tubes were tied into either end and the loop was well washed out to remove all contents. Into the loop was introduced either glucose or glucose with 10 p.c. alcohol. In these experiments the periods were half-hours.

## RESULTS.

## Disappearance of glucose from small intestine.

Exp.	1st half-hour glucose with alcohol (grm.)	2nd half-hour glucose alone (grm.)
I	0.813	0.541
II	0.733	0.46
III	0.645	0.432
IV	0.88	0.81
V	0.82	0.66
Mean	0.78	0.59

These experiments were complicated by the fact that in some cases tape-worms were not completely removed. No great stress can therefore be laid upon the precise values of the estimations; they can only be regarded as indicative.

It is better perhaps to speak of the disappearance of glucose than its actual absorption. J. Mellanby<sup>(13)</sup> has pointed out that etherisation and decerebration will cause variable fluctuations of the blood sugar level. No reliance therefore could be placed on simultaneous estimations of the blood sugar as far as relationship between disappearance of sugar from the alimentary canal and variations in the amount in the blood were concerned.

It seems however permissible to state that alcohol influences the rate of disappearance of glucose from the stomach, and in work which is now proceeding the effect of alcohol is being further studied under conditions in which etherisation and decerebration are eliminated and the results so far indicate that the disappearance corresponds definitely to absorption into the blood.

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# THE IDENTICAL SOURCE OF WORK AND HEAT IN MUSCULAR CONTRACTION.

BY W. HARTREE.

*(From the Physiological Laboratory, Cambridge.)*

It is commonly assumed that the heat liberated by a stimulated muscle, and the work done by it when it is allowed to shorten against a load, are derived from the same source, namely the anaerobic formation of some substance *B* from some other substance *A*. It is very probable that *B* is potassium lactate, while *A* is either glycogen, or more immediately perhaps a compound of sugar with phosphate. Following this anaerobic breakdown, in the presence of oxygen *B* reverts to *A* while a certain amount of glycogen is oxidised, the net result of the whole cycle being heat liberated, work done, oxygen used, carbon dioxide produced, and a certain amount of glycogen burnt. In the experiments on which this chemical picture is based no attention has been paid to the question of whether the energy liberated by the muscle has appeared entirely as heat, or partly as heat, partly as mechanical work. In the majority of experiments the conditions were such that comparatively little external work was done. Now it is conceivable, though on general grounds unlikely, that the source of heat may not be the same as the source of work when the muscle is allowed to shorten against a load. The tacit assumption of their identity might conceivably lead to serious error in building up the theory of muscular contraction. In the experiments to be described one characteristic of the energy liberated by a working muscle has been compared with the same characteristic in a muscle contracting isometrically, and within the limits of experimental error an identity has been found. If work is derived from the same chemical source as heat, then the ratio of the total recovery heat to the total initial energy should be the same in an isometric contraction (initial energy all heat) as in one where a considerable amount of work is performed (initial energy partly heat and partly work).

The following experiments on the magnitude of the recovery heat were performed on frogs' sartorius muscles at 18° C. in oxygen during the winter months. The energy liberated in recovery is expressed



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hand it is obvious that the recovery heat in the working muscle has quite a different value when expressed as a fraction, not of the energy liberated in the initial phase, but of the heat alone.

The ratio of work done to total initial energy is low, as seems invariably to be the case with frog's muscle. In the last six experiments the work amounts on the average to 20 p.c. of the whole initial energy. If, however, the chemical breakdown producing work required a different amount of energy for its reversal from the breakdown producing heat, the work done, namely 20 p.c., might be large enough to cause a measurable effect on the observed recovery heat. On the whole, therefore, the probability is strengthened that the process producing work and that producing heat are both followed by the same kind of recovery reaction, indeed that they are chemically speaking the same.

In one experiment the work done was negative, which means that the muscle was stretched out during stimulation. Several experiments of this kind were tried, but in this one case alone was the third series similar to the first: in the rest the muscle was probably stretched permanently to some extent, and so its position on the thermopile was not the same for the final as for the original set. In this particular experiment, when work was done, the observed heat was 376 and the total energy liberated by the muscle 254. In spite of the large amount of work (negative) the recovery heat was exactly the same fraction of the heat in the isometric case as it was of the total net energy in the working case.

It will be noticed that the values given for the recovery heat as a fraction of the initial energy are lower than in experiments previously recorded: in very few cases was the value 1.5 attained, although this has often been observed before (2, 3): in most cases it was appreciably less, the average of the last six experiments being 1.28. The actual figure, however, probably depends upon the magnitude of the delayed anaerobic heat (see Furusawa and Hartree (4)): if this be absent, as recent experiments by A. V. Hill (5) show to be the case in a series of separate twitches, the ratio of recovery to initial heat is only 1.07. Hartree and Liljestrand, for a short tetanus of a tortoise's leg muscle, found a value of about 1.2 (6). The actual figure, however, and the differences between the corresponding numbers in the different experiments, have no effect on the argument given above: the comparison is between the isometric and the working cases in each individual experiment.

as a fraction of the initial energy, namely, of the observed heat in the isometric case and of the observed heat plus the work done when the muscles are allowed to shorten against a load. For further comparison, in the working muscle the recovery heat is expressed also as a fraction of the initial heat as distinguished from the initial energy. In every experiment two or three records were taken for isometric contractions, then two or three when doing work, and a third set of two or three isometric again, each recovery observation being continued 18 to 20 minutes to make sure of getting a good zero for the next observation. If the final set of observations did not agree well with the original set, the experiment was discarded as being not wholly reliable.

In the first four experiments of Table I the work done consisted of lifting a known weight for a measured height, the weight being left at the top and the muscle returning to its original position under a small load on relaxation. In the other six experiments the work was automatically recorded by tension-length diagrams, as described by Levin and Wyman(1). The latter method is more reliable, as there was an unknown amount of friction in the former. In the first four experiments it will be seen that isometric recovery gives a rather smaller result than recovery after work, whereas in the last six the two are practically equal and the individual differences are much smaller. Taking particular account of the last six experiments, it is seen that in the isometric case the recovery heat is practically the same fraction of the initial energy as in the case of the muscle doing work, the mean 1.28 for the former being indistinguishable from the mean 1.27 for the latter. On the other

TABLE I. Comparison of recovery heat-production in isometric and working contractions.

Duration of stimulus secs.	Iso-metric heat	When doing work			Recovery: fraction of			"Initial" efficiency p.c.
		Observed heat	Work	Energy	Heat: iso-metric	Energy: working	Heat: working	
0.10	188	186	50	236	0.83	0.98	1.24	21
0.10	200	187	38	225	1.12	1.24	1.49	17
0.15	143	146	34	180	1.37	1.41	1.74	19
0.15	280	279	55	334	0.72	0.83	0.99	16
0.15	218	191	34	225	1.21	1.23	1.45	15
0.15	133	132	30	162	1.56	1.51	1.85	19
0.15	131	120	43	163	1.38	1.33	1.78	26
0.15	213	195	74	269	1.12	1.15	1.59	27
0.30	278	270	45	315	1.18	1.16	1.35	14
0.30	281	264	64	328	1.24	1.25	1.55	20
		Mean of all	...	...	1.17	1.21	1.50	19
		Mean of last 6	...	...	1.28	1.27	1.59	20

Heat and energy are given in gram-centimetres.

# ON THE FLUCTUATION IN THE COMPOSITION OF THE ALVEOLAR AIR DURING THE RESPIRATORY CYCLE IN MUSCULAR EXERCISE.

BY R. S. AITKEN AND A. E. CLARK-KENNEDY.

*(From the Medical Unit, The London Hospital.)*

## INTRODUCTION.

THE measurement of the circulation rate in man has been attempted by various methods, none of which has come into general use. The problem is important in physiology, and possibly more so in medicine, where physiological explanations are sought for the phenomena of disease. For that reason, among others, the recently devised ethyl iodide method of measuring the circulation rate published by Yandell Henderson<sup>(5)</sup> in 1925, has attracted considerable attention. In this method the subject breathes a certain concentration of ethyl iodide vapour for a given time, during which the amount of ethyl iodide taken up by his blood and the concentration of the vapour present in his alveolar air are determined; from these results, given a knowledge of the partition coefficient of ethyl iodide between blood and air, the circulation rate can be calculated. In such an experiment, the reliability of the method of sampling alveolar air is obviously of fundamental importance. Yandell Henderson uses an automatic modification of Krogh's Copenhagen method, whereby in successive expirations small samples are taken from the last portions of air expired, and it is assumed that the composition of the mixed sample obtained in this way is the same as the composition of alveolar air. Alveolar air, however, varies in composition during the respiratory cycle; in expiration its  $\text{CO}_2$  increases and its oxygen decreases, while the opposite changes take place in inspiration. For calculation of the circulation rate it is necessary to know the average alveolar composition throughout the experimental period, which comprises many respiratory cycles. How far the composition of Yandell Henderson's sample approximates to that average is not clear. The fractions of his sample are withdrawn from the alveoli at the same phase of successive respiratory cycles; that phase may coincide with the moment at which

## SUMMARY.

1. The recovery heat-production of a frog's sartorius muscle in oxygen has been compared with the total initial energy in the two cases, (a) isometric, and (b) doing work to the extent of about 20 p.c. of the initial energy. The recovery heat is the same fraction of the initial energy in either case. It is a considerably greater fraction of the initial heat in the working case than in the isometric one.

2. From this it is argued that the process which produces work in a shortening muscle has to be followed by the same recovery process as that which produces heat alone in an isometric contraction. Both the work and the heat probably come from the same reaction, namely the formation of lactate from its precursor.

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## DESCRIPTION OF APPARATUS.

The apparatus consists of the following parts:

- I. A Central Chamber, through which the subject breathes; in its walls are six openings, fitted with electrically operated valves, leading into six small rubber bags.
- II. A System of Switches which operate the valves automatically.
- III. A Recording System, which simultaneously registers the action of the valves and the breathing of the subject on a revolving drum.
- IV. A Douglas Bag.

These will now be described in detail.

I. *The Central Chamber* is an oblong air-tight brass box which is shown in longitudinal section in Fig. 1. When the subject breathes

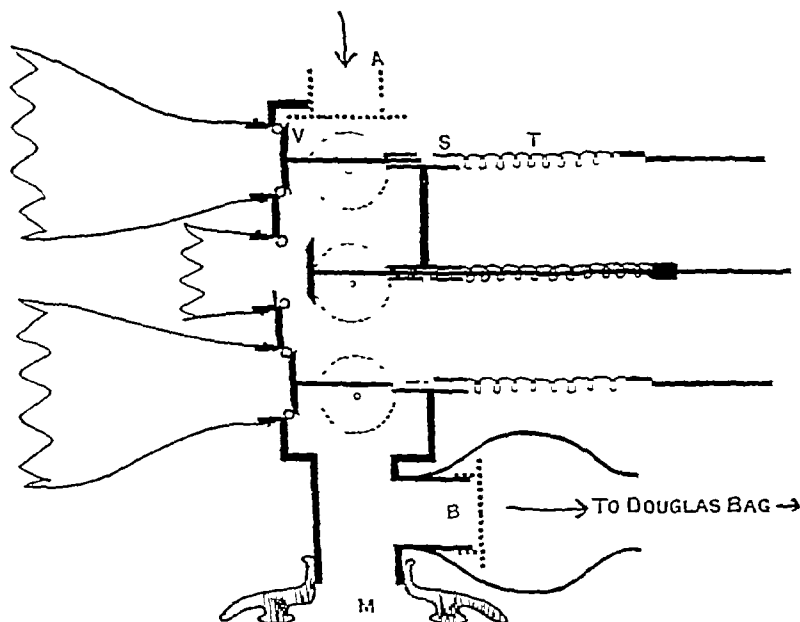


Fig. 1. Diagrammatic longitudinal section of the central chamber of the apparatus.

through the mouthpiece *M*, air enters at *A* through a Rosslyn valve fixed by adhesive into a hole in the brass, and passes out at *B* through another similar valve and a piece of corrugated rubber tubing into the Douglas bag. Three of the six openings in the sides of the chamber are shown on the left hand side of the figure, leading into short lengths of



the fluctuating composition of the alveolar air is passing through its mean, but it cannot be proved to do so until the extent of the fluctuation is accurately known. It may be that in some types of breathing (*e.g.* at rest) the coincidence is good, while in others (*e.g.* during work) there is a discrepancy. The aim of the work to be described in this paper was to obtain some definite information about the degree to which the composition of alveolar air varies throughout the respiratory cycle, and if possible to compare its average composition with that of the last portion of the expired air which constitutes Yandell Henderson's alveolar sample. All the observations were made during muscular exercise, because the amount of variation in the alveolar concentrations, and the discrepancy (if any) between their average values and those of the Yandell Henderson sample, are both likely to be greater when the breathing is deep and the respiratory exchange increased.

It would be very difficult to follow the changes in the alveolar air by a direct method, such as taking Haldane-Priestley samples at different phases of the respiratory cycle, because every sampling would disturb the rhythm of the breathing. An indirect method, however, is applicable during expiration, for if the variation in the composition of the air *passing out of the mouth* in a single expiration is observed, it is possible to deduce what changes must have occurred in the alveolar air to give rise to the observed changes in the expired air. This was the method adopted. For observing the changes in the expired air some form of apparatus was required which would receive a single expired breath, and divide it up into a number of separate successive portions suitable for measurement and analysis. This, moreover, must be done without offering any obstruction to the expiration, and without altering its rate or its volume. Such an apparatus was devised, based on a system of automatically operated valves which directed successive portions of an expired breath into six small rubber bags. It was made of a size large enough to accommodate breaths of  $1\frac{1}{2}$  to  $3\frac{1}{2}$  litres, which might be given by a subject doing moderate muscular work. In the following pages the apparatus and the experiments carried out with it will be described first. Then the results will be given, together with the deductions that may be drawn from them, including certain conclusions as to the size and the nature of the respiratory dead space.

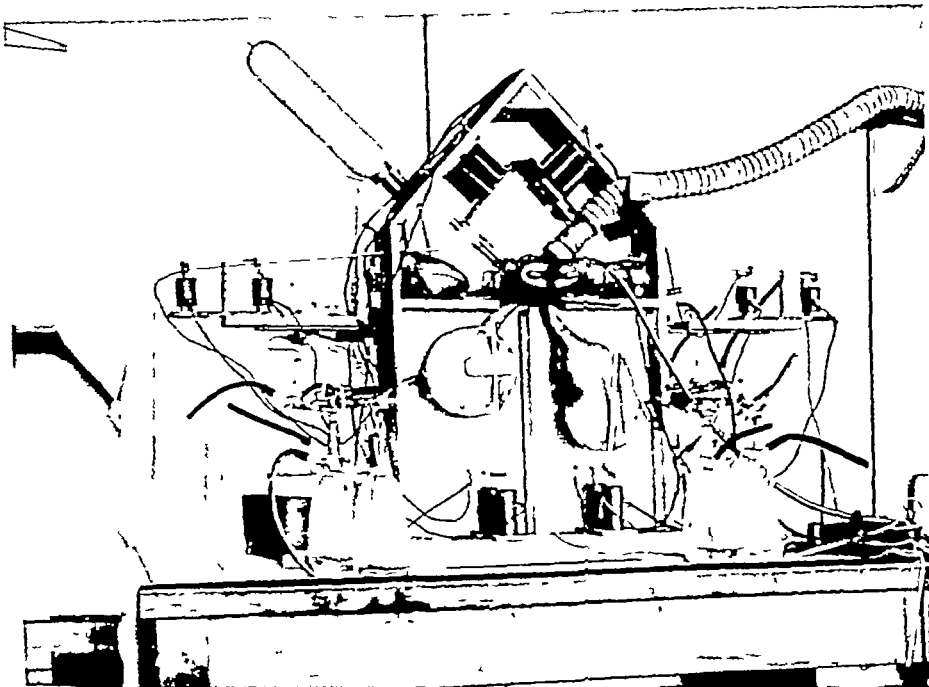


Fig 2. Photograph of the apparatus showing the central chamber, electromagnets, mercury switches, and small rubber bags, mounted in a wooden frame, together with the sampling bottles in position.

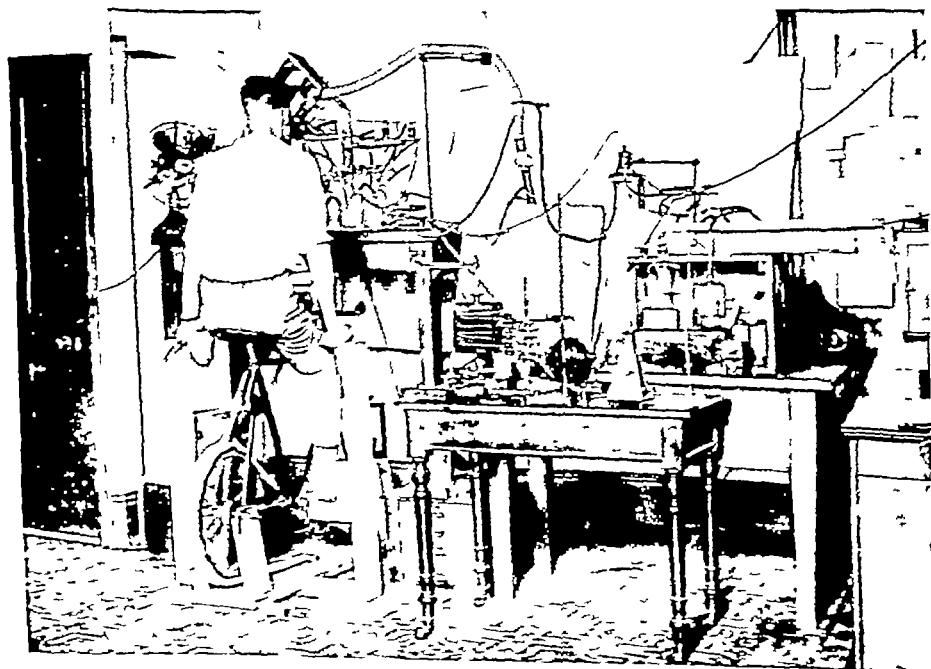


Fig 3. Photograph showing the arrangement of the whole apparatus for an experiment.

brass tubing to which are fitted the necks of collapsible thin rubber bags of about 500 c.c. capacity. Each opening is provided with a valve *V*, made of a disc of metal, bevelled at the edge, and carried on the end of a thin metal rod. The valve seating is a ring of narrow rubber tubing fixed with adhesive to the margins of the opening in the brass wall; when the valve is pressed on to it, wet or dry, it makes an air-tight contact; the rubber must be kept free from grease. The rod occupying the disc passes through a close-fitting and well-greased brass sleeve, *S*, soldered into the opposite side of the chamber. Its other end is attached to an electromagnet which opens the valve (the middle one of the three valves is shown open), while a spring *T* keeps the valve shut when the magnet is not in action. These three valves and their sleeves occupy two opposite sides of the chamber, which is square in cross section; the other two sides are similarly occupied by the other three valves (shown as dotted circles in the figure) and their sleeves.

Fig. 2 is a photograph showing the chamber with its six rubber bags, together with the magnets and their connections, mounted in a wooden frame. Each bag has a narrow rubber side-tube leading through a glass four-way-piece to three gas-sampling bottles of the kind described by McCann and Hannan and figured by Clark-Kennedy and Owen<sup>(1)</sup>. Three of them are sufficient to hold the contents of one bag; they contain a mixture of equal volumes of glycerine and saturated sodium chloride solution, over which expired air can be kept for 6 hours without loss of  $\text{CO}_2$ . The magnets are operated by a 240-volt current from the mains passing through a resistance of 120 ohms.

II. *The System of Switches.* Each rubber bag is associated with a double mercury switch, seen in Fig. 2 and shown diagrammatically in Fig. 3. Attached loosely to the surface of the bag is a hinged aluminium rod *R*, carrying a transverse ebonite arm with two steel pins dipping into ebonite cups of mercury. When the bag is collapsed, contact is made in the cup *M*, which is in the circuit of the electromagnet controlling the valve of the same bag. When the bag is filled with air it takes up

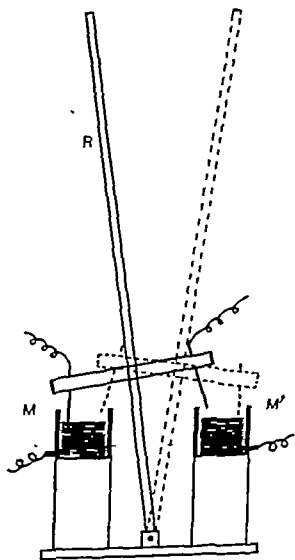


Fig. 3. Diagram of a mercury switch.

the position shown by the dotted lines: contact is broken at *M*, whereupon the valve of this bag closes: contact is made at *M'* in the other mercury cup which is in the circuit of the magnet that opens the valve of the next bag in the series. In this way each bag as it becomes full closes its own valve and opens that of its neighbour. In an experiment, this process is begun by switching the main current into the circuit of the first electromagnet, and if air is blown into the central chamber it then continues automatically down the series of six bags until the filling of the last one short-circuits the current.

III. *The Recording System.* In the circuit of each magnet is an ordinary signal-marker, which records on a smoked drum the time during which that magnet was in action. A pressure record of the breathing is also taken on the drum by means of a polygraph tambour connected through fine rubber tubing with the inside of the central chamber: this is very sensitive and gives an accurate record of the time occupied by inspiration and expiration respectively. A Jacquet clock marks fifths of seconds.

IV. *The Douglas Bag* holds up to 70 litres of air. It is connected with the expiration valve of the central chamber through corrugated rubber tubing and a 3-way tap, so that the expired air may either be collected or be allowed to escape as desired.

#### DESCRIPTION OF EXPERIMENTS.

The apparatus was designed to accommodate the rapid and deep breathing of muscular exercise, and care was taken that no air-way should be less than 0.45 sq. in. in cross section. At the same time the dead space of the valves, which is the whole capacity of the central chamber, was kept as small as possible, and for this purpose its superfluous corners were filled with sealing-wax: this instrumental dead-space is 160 c.c. If an expired breath is to be collected in the six bags of the apparatus, it is obvious that at the beginning of expiration these 160 c.c. will contain fresh air, which, during expiration will be swept with the expired air into the bags. In order to find out how quickly this instrumental dead space is washed out, air containing a known uniform concentration of  $\text{CO}_2$  was blown into the apparatus from a Douglas bag. In this as in all subsequent experiments the valves furthest from the mouthpiece were the first to open, so that the dead space might be washed out as quickly as possible. The air in the Douglas bag and in the small bags was then analysed, and in one experiment gave the following figures:



but would not prejudice the results so long as the breathing during the time of collection of expired air was uniform.

Exactly at the end of the ninth minute of work the 3-way tap of the Douglas bag was turned, and from then till the end of the tenth minute the expired air was collected in the Douglas bag, while the respirations were counted. About half-way through the tenth minute, during an inspiration, the expired air tube was suddenly clamped just beyond its valve, and at the same moment the current to the apparatus was switched on. This caused the valve of the first small rubber bag to open, so that the succeeding expiration passed first into this bag and then into the other five in succession as the automatically operated valves came into action<sup>1</sup>. During the following inspiration the current was switched off, and the clamp on the expiration tube released, allowing the expired air during the rest of the minute to pass into the Douglas bag as before. By this whole procedure a single expiration, in the middle of the tenth minute, was diverted in six distinct and successive portions, into the six small bags. This breath will be called the "divided expiration." Meanwhile an assistant had started and stopped the rapidly revolving drum of the recording system at such times as to give a record of the operation of the valves and the duration of the divided expiration. At the end of the tenth minute, the tap of the Douglas bag was closed, and the subject ceased work.

Immediately after that the contents of the six small bags were quickly sucked into the sampling bottles; it had been found out beforehand that if this was carried out inside 2 or 3 minutes, the absorption of  $\text{CO}_2$  by the rubber walls of the bags was negligible. The expired air from the six small bags, and the expired air in the Douglas bag, were then measured and analysed. Measuring in the case of the small samples was done in a 700 c.c. burette over glycerine and salt mixture, and the samples were returned to the sampling bottles for analysis. From the air in the Douglas bag two samples were taken for analysis, and the rest measured in a spirometer. Analyses for oxygen and  $\text{CO}_2$  were done in duplicate on two Henderson-Haldane burettes; when agreement was not satisfactory they were repeated.

The drum records were measured and from them were calculated the times of opening and closing of the six valves, and the time of beginning

<sup>1</sup> At the end of the expiration the sixth bag (which was rather larger than the others) might be only partly full, in which case its valve might remain open, but no air could escape from it again because a one-way rubber spear-valve was fitted just inside its neck.

Douglas bag	...	4.18 p.c. CO <sub>2</sub>
1st bag	...	2.66 „
2nd bag	...	4.09 „
3rd bag	...	4.16 „
4th bag	...	4.17 „
5th bag	...	4.18 „
6th bag	...	4.17 „

A second experiment gave a similar result. The figures show that the washing out is completed by the time the second bag is full.

: After this preliminary observation, two sets of experiments were carried out on two subjects. In all these the object was to obtain a breath during muscular exercise, divide it into 5 or 6 successive portions, and determine the changes in gaseous composition from one to another. Six experiments were performed on the subject J.K.M. and eleven on the subject R.S.A. The procedure was essentially the same in them all though there were some minor variations in the first set. The following are the details of a typical experiment.

The subject sat erect on a bicycle ergometer, and the apparatus was so placed that he could take the mouthpiece in his mouth without discomfort or hindrance to his breathing. The general arrangement is shown in Fig. 4. He inspired from the atmosphere through the central chamber, and expired again to air through the 3-way tap on the tube leading to the Douglas bag. As soon as he was comfortable he began to ride the bicycle, at 72 turns of the pedals per minute, keeping time with a metronome. The load on the ergometer was constant for a given experiment, but was varied in different experiments in order to give different volumes of tidal air. The work was continued for 10 minutes, and a fan was used to keep the subject cool in the second half of that time. If a person riding an ergometer under these conditions pays no attention to his breathing, he involuntarily breathes in time with his pedalling, taking one breath to every two, three, or four turns of the pedals, according to the severity of the work, and probably changing at intervals from one ratio to another if his respirations become uncomfortably deep or shallow. In these experiments such a sudden change during a critical period was to be avoided; so the ratio of breathing rate to pedalling rate was decided on beforehand, and the subject consciously kept to it. Both the rate of breathing and the rate of pedalling were so chosen (after numerous trials) as not to involve excessive over- or under-breathing; a minor degree of one or the other was likely to occur,

however, the  $\text{CO}_2$  concentration in the actual air expired does not change in this intermittent stepped fashion but continuously, and should be represented by a smooth curve. If this smooth curve is to be an accurate representation, it is necessary that the area of the part of it corresponding to each bag shall be equal to the area of the rectangle corresponding to that bag, since each of these areas is equivalent to the amount of  $\text{CO}_2$  in the bag. To achieve this in practice, the smooth curve was first drawn "by eye" through the tops of the rectangles (plotted on ruled paper) and the areas were compared by counting squares in the figures  $x$  and  $x'$ ,  $y$  and  $y'$ , etc.; if necessary, the curve was then altered until  $x = x'$ ,  $y = y'$ , and so on. In this way, by trial and error, a reasonably smooth curve was arrived at, which satisfied the condition stated above. In every experiment there was found to be very little latitude in the placing of this curve—the six rectangles fixed it remarkably closely. Curves drawn in this way are more accurate than smooth curves drawn merely through the mid-points of the tops of the rectangles.

Eleven experiments of this kind were performed on the subject R.S.A. and six similar ones in the earlier set on the subject J.K.M. Three experiments were discarded on account of irregularities in the breathing of the subject or in the operation of the automatic switches. There remain five on J.K.M. and nine on R.S.A. The results of these will now be given and discussed.

## RESULTS AND DISCUSSION.

### I. *The curve of $\text{CO}_2$ concentration in an expired breath.*

The smooth  $\text{CO}_2$  curves obtained in the manner just described in the fourteen experiments under discussion, are shown in Fig. 6, and the general data of the experiments are given in Table I. The curves represent the results of all the experiments done that were not demonstrably defective; they are all reproduced because on their combined evidence rests the principal conclusion of this paper, namely, that the  $\text{CO}_2$  curve of the expiration given in these experiments by a subject doing moderate muscular work on an ergometer has a typical shape. Beginning at zero, every curve exhibits an S-shaped rise in the  $\text{CO}_2$  concentration up to about 1000 or 1200 c.c.; thereafter, in nine cases it proceeds as a straight line, and in the remainder its deviation from a straight line is so small that it can be ascribed to slight irregularities in breathing, or to minor experimental defects. Furthermore, this straight part of the curve, in every case but one, is not horizontal but slopes gently upwards. The



of the next inspiration, taking the beginning of the divided expiration as zero.

The gas volumes, measured moist at room temperature, were recalculated for 37° C., moist. The oxygen intake, CO<sub>2</sub> output, and respiratory quotient were calculated for each bag separately, for the whole divided breath, and for the expired air of the whole tenth minute.

Finally the CO<sub>2</sub> curve for the divided breath was plotted by the method illustrated in Fig. 5. The whole volume of the breath was set

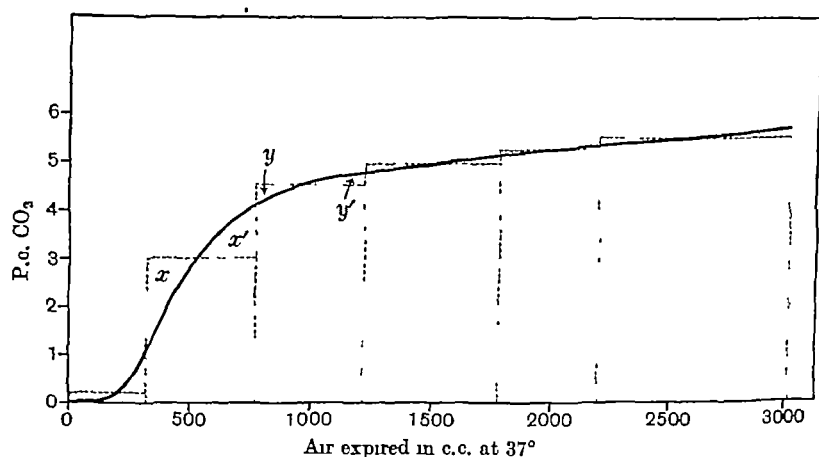


Fig. 5. Diagram showing the method of plotting the CO<sub>2</sub> curve of an expired breath (Exp. 12).

Bag No.	Volume in c.c.	P.c CO <sub>2</sub>
1	324	.22
2	444	2.97
3	450	4.52
4	562	4.98
5	417	5.27
6	829	5.53

out along the abscissa, and subdivided into six parts corresponding to the volumes of the six portions of the breath in the small bags, the first portion to be expired being placed on the left; on each of these subdivisions as base a rectangle was drawn, with its height representing the concentration of CO<sub>2</sub> in the corresponding bag. The area of each rectangle (volume  $\times$  CO<sub>2</sub> concentration) represents the actual amount of CO<sub>2</sub> in the corresponding bag, while the combined areas of the six rectangles will be equivalent to the total amount of CO<sub>2</sub> in the whole breath. The upper limit of the combined area is a "stepped" curve, which represents literally the concentrations found in the six portions;

typical curve, therefore, is made up of two parts: the first rises from zero with a double (S-shaped) inflection, and is continued into the second, which is a straight line sloping slightly upwards. (Fig. 5 shows a good example on a larger scale.)

The explanation of this constant shape of the curve seems fairly clear. The whole curve represents the changing  $\text{CO}_2$  concentration in the air that is entering the small bags of the apparatus, some of which has come up from the pulmonary alveoli (alveolar air), and some from the bronchi, the trachea, the mouth, and the central chamber of the apparatus, that is, from the dead space of the subject and of the machine (dead space air). The S-shape of the first part is due to the mixing of alveolar air rich in  $\text{CO}_2$  with dead space air that contains little or no  $\text{CO}_2$ . The alveolar air tends to come up in axial streams, moving more rapidly in the axes of the respiratory tubes, and more slowly next their walls where it is delayed by friction; hence the  $\text{CO}_2$  appears in the small bags of the apparatus before the dead space is completely "washed out," and the curve obtained is not rectangular but doubly inflected. The effect of this dilution of alveolar air with fresh air is however a progressively diminishing one; it explains the S-shape of the first part of the curve, but it cannot account for the slope of the second straight part, which is a uniform one. That uniform upward slope in the second part means that *the air which left the alveoli* contained a steadily increasing percentage of  $\text{CO}_2$  as expiration progressed, for, when the dead space has once been washed out, air will pass from the alveoli to the small bags of the apparatus almost unchanged; admittedly the axial stream phenomenon still operates, but the sliding of one portion of air past another, which this involves, can have little effect on the slope of the curve when that slope is not great. The same argument applies to such eddies as may occur in the chamber of the apparatus, and it has already been seen that about 800 c.c. of air (contents of first two bags) are sufficient completely to wash out this instrumental dead space. Finally, if it be granted that the air in the alveoli is uniformly mixed, then the straight part of the experimental curve represents the  $\text{CO}_2$  concentration in alveolar air, and it slopes up because in the second part of expiration that concentration is increasing at a uniform rate.

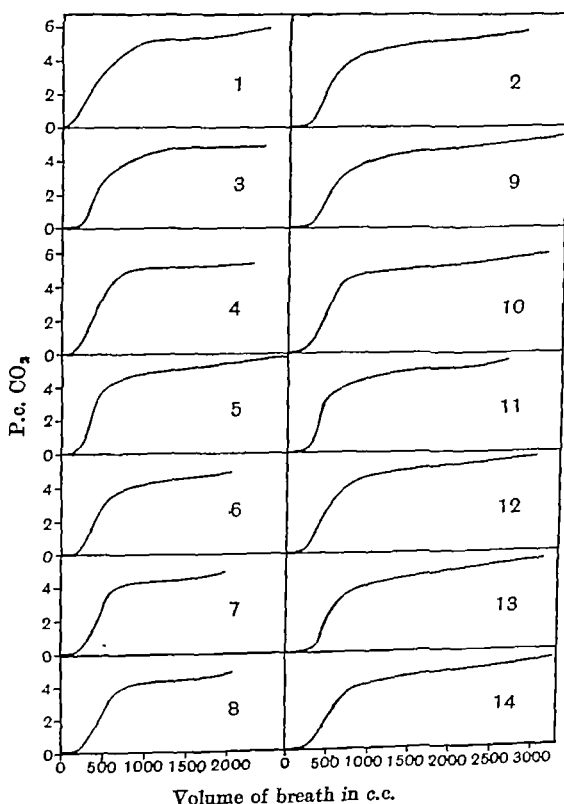
## II. *The physiological dead space in deep breathing.*

An attempt will be made later to define the physiological factors which influence the slope of the alveolar  $\text{CO}_2$  curve during the whole of expiration. So far the actual curve has been obtained directly in the

TABLE I. General data of Experiments 1 to 14.

Exp. No.	Subject	Duration mins.	Ergo-meter tension lbs.	Rate of pedalling per min.	Rate of breathing per min.	Last minute*			Volume divided breath c.c. at 37°
						CO <sub>2</sub> output c.c. at 37°	O <sub>2</sub> intake c.c. at 37°	Tidal air c.c. at 37°	
1	J.K.M.	5	6	80	20	1549*	1628*	3195	2447
2	J.K.M.	5	5	80	20	1480*	1428*	3030	2830
3	J.K.M.	5	4	88	22	1140*	1208*	2430	2501
4	J.K.M.	5	4.5	88	22	1050*	1091*	2100	2293
5	J.K.M.	8	4.5	88	22	2433	2418	2228	2679
6	R.S.A.	10	6	72	36	2343	2282	1890	2038
7	R.S.A.	10	6	72	36	2358	2147	1940	1967
8	R.S.A.	10	6	72	36	2234	2051	1931	2079
9	R.S.A.	10	6	72	18	2117	2062	2880	3285
10	R.S.A.	10	6	72	18	1696	1702	2370	3103
11	R.S.A.	10	6	72	18	1771	1770	2356	2665
12	R.S.A.	10	8	72	18	2105	2148	2760	3026
13	R.S.A.	10	8	72	18	2652	2233	3520	3123
14	R.S.A.	10	9	72	18	2597	2470	3410	3260

\* Last half minute in Nos. 1, 2, 3 and 4.

Fig. 6. The CO<sub>2</sub> curves obtained in Experiments 1 to 14.

a method for determining the physiological dead space in deep breathing during work.

The physiological dead spaces have been determined by this method from all the curves shown in Fig. 6. In practice it was found easier to make the areas equal by algebraical calculation instead of actually counting squares; the dead space was regarded as containing  $\cdot 03$  p.c.  $\text{CO}_2$ .

In the five experiments on the subject J.K.M. the physiological dead space was found to vary from 228 c.c. to 417 c.c. (measured moist, at  $37^\circ \text{C}$ ., and prevailing barometric pressure), but it bore no definite relation to the volume of the tidal air. Those experiments, as the curves show, were in other respects more irregular and less satisfactory than the subsequent ones on the subject R.S.A. In the latter (Nos. 6 to 14), the dead space varied from 283 c.c. to 392 c.c., and bore a very definite relation to the volume of the tidal air<sup>1</sup>, as will be seen in Table II and

TABLE II. Dead space and tidal air in Experiments 6 to 14.

Exp. no.	Average tidal air in tenth minute c.c. at $37^\circ$	Physiological dead space c.c. at $37^\circ$
6	1890	283
8	1930	290
7	1940	280
11	2356	287
10	2371	295
12	2760	327
9	2880	361
14	3410	378
13	3520	392

Fig. 8. As the size of the breath increases, the dead space increases proportionally over the range covered by the experiments, *i.e.* from 1890 c.c. of tidal air to about  $3\frac{1}{2}$  litres. It is not clear, however, whether the rate of breathing has a separate influence on the dead space, over and above that of the depth, although it is suggestive that the three points 6, 7 and 8 are a little out of the line established by the remainder; 6, 7 and 8 are the experiments where the breathing was 36 per minute, while in the rest it was 18 per minute.

These results may be compared on the one hand with those of Haldane and Douglas, and on the other with those of Krogh and

<sup>1</sup> "Tidal air" means average tidal air for the last minute of the experiment. The whole volume of the divided breath sometimes differed from this (see Table I), but as the difference was due to the divided expiration having been cut short too soon or unduly prolonged, rather than to an irregularity in the inspiration that preceded it, it is more reasonable to seek a relationship between the dead space and the tidal air than between the dead space and the volume of the divided breath.

latter half or two-thirds of expiration, and it shows that whatever those factors are, they act together during that time in a uniform manner to produce a uniform upward slope. It seems likely, then, that they will also act together in the same uniform manner during the earlier part of expiration, when the experimental curve is not identical with the alveolar air curve, but is modified by the admixture of the dead space air. If that be so, the alveolar air curve for the earlier part of expiration can be reconstructed by prolonging the straight part of the experimental curve to the left, as has been done by the dotted line *BD* in Fig. 7. The

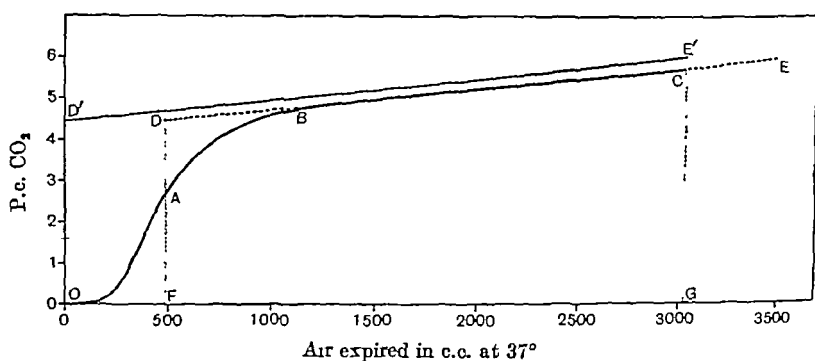


Fig. 7. Diagram to show the method of deducing the alveolar  $\text{CO}_2$  curve and the dead space from the experimental  $\text{CO}_2$  curve (Exp. 12).

The flat part *BC* of the experimental curve *OABC* is prolonged to the left to *D* until the area *FDCG* equals the area *OABCG*, *DF* and *CG* being perpendicular to *OG*. *OF* then represents the volume of the dead space. *DC* is prolonged to the right to *E*, over a distance equal to *OF* (when measured horizontally). The whole line *DE* then represents the changing concentration of alveolar  $\text{CO}_2$  throughout expiration.

line *DC* now represents the  $\text{CO}_2$  concentration of all the air that left the alveoli and also reached the small bags of the apparatus. That air is smaller in volume than the total expired air collected in the bags, the difference being the volume of the dead space, but it must contain the same amount of  $\text{CO}_2$  as the total expired air, because the dead space air contains practically none. Since amount of  $\text{CO}_2$  is represented in Fig. 7 by area (volume of air  $\times$   $\text{CO}_2$  concentration), this fact enables the point *D* to be fixed, by the simple procedure of making the area of the figure *FDCG* (representing amount of  $\text{CO}_2$  in the air from alveoli) equal to that of the figure *OABCG* (representing amount of  $\text{CO}_2$  in expired air). When that is done the distance *OF* represents the total dead space, instrumental plus physiological. This reconstruction, then, affords

quantities to raise the  $\text{CO}_2$  concentration in the alveolar air well above what it is at the beginning or at the end (as the case may be) of an ordinary expiration. Hence, in exercise, the Haldane-Priestley alveolar air contains more  $\text{CO}_2$  than average alveolar air, and the dead space calculated from it is too high. As the amount of work increases, the  $\text{CO}_2$  output increases, while the time taken to give the Haldane-Priestley sample remains the same; hence the error becomes greater, and the dead space appears to rise with increasing tidal air more rapidly than is really the case.

Krogh and Lindhard(7), on the other hand, maintain that the dead space at rest is of the order of 100 c.c. and that with deep breathing (at rest also) its maximum rise is to about 200 c.c. They based this conclusion on the results they obtained with Siebeck's hydrogen method, or modifications of it; their views on the dead space at rest are discussed fully by Haldane(4). They also give, however, the results of a few experiments not unlike those described in this paper, and performed during work. Their subject expired into a recording spirometer, which, by operating electric contacts, caused two or three samples of air to be taken from between the subject's teeth into evacuated mercury sampling tubes, in the course of one expiration. A third or fourth sample was taken of the very end of the breath, from the expiratory tube, just beyond the valve. The samples were analysed, and the corresponding volumes obtained from the spirometer record. Four experiments are mentioned; three of them have three samples each, the fourth has four and its results are plotted,  $\text{CO}_2$  p.c. in sample against volume. All four indicate that during the latter part of the breath, the  $\text{CO}_2$  p.c. rises along a straight line; the slope of the line is steeper than in our experiments, but this was probably because the breathing was much slower. The fourth is the only one in which the  $\text{CO}_2$  percentage in the expired air is given, and is therefore the only one in which the dead space can be even approximately calculated by the reconstruction method given above. In that experiment, using the line which Krogh and Lindhard have drawn through their four somewhat irregular points, it works out at something in the neighbourhood of 100 c.c. This is in accord with their theory, based on the hydrogen experiments, that the dead space in deep breathing is not greatly increased. It is, however, a single observation (or only very weakly supported by the other three), and a less complete one than any single one of the experiments in this paper. Moreover, the respiratory quotients for the four samples in this experiment are .64, .65, .66, .61, values which seem surprisingly low for a man doing muscular work, and absorbing 1830 c.c. oxygen per minute. It is possible that, at the

Lindhard. Haldane and Douglas<sup>(3)</sup> calculated the CO<sub>2</sub> dead space in Douglas both at rest and at various speeds of walking, determining the

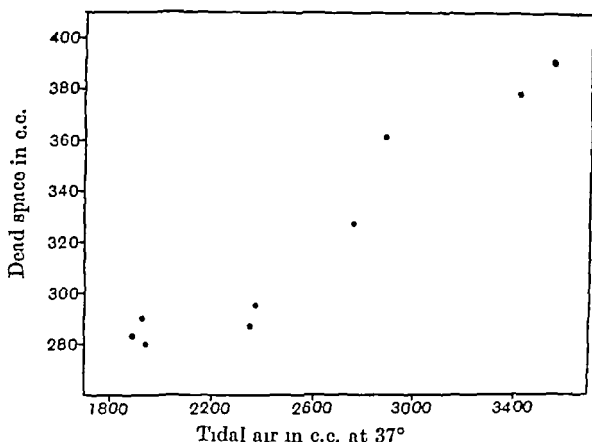


Fig. 8. The relation of dead space to tidal air in Experiments 6 to 14.

average tidal air, the CO<sub>2</sub> in the expired air, and the CO<sub>2</sub> in the alveolar air. The alveolar air was obtained by Haldane and Priestley's method, taking the mean of inspiratory and expiratory samples. The resulting dead space was 213 c.c. (moist, at 37° C. and prevailing barometric pressure) at rest for a tidal air of 457 c.c., and at the various levels of work it increased very regularly with the increasing tidal air up to 675 c.c. for a tidal air of 3145 c.c., when the subject was breathing 60.9 litres of air and consuming 2543 c.c. of oxygen (at N.T.P.) per minute. Our figures in the first place are considerably lower than these; *e.g.* in Exp. 14 when the ventilation was 63.2 litres and the oxygen consumption 2047 c.c. per minute, the dead space was only 391 c.c. for a tidal air of 3410 c.c. Further, while our figures confirm the conclusion of Douglas and Haldane that the dead space does increase with increasing depth of breathing, they indicate a much less rapid increase (about 8 c.c. per 100 c.c. tidal air) than do Douglas and Haldane's figures (about 19 c.c. per 100 c.c. tidal air). These two discrepancies appear to be due to one cause, which was pointed out by Krogh and Lindhard<sup>(6)</sup> and recognised by Douglas<sup>(2)</sup>, namely, that the Haldane-Priestley method of sampling alveolar air is not reliable when the CO<sub>2</sub> output is increased by muscular work. It takes time to make the deep expiration involved, and during that time CO<sub>2</sub> can pass from the rapidly circulating blood in the lungs into the rapidly diminishing volume of air in the alveoli, in sufficient

influx of  $\text{CO}_2$ ) would cause the alveolar  $\text{CO}_2$  to rise, and to rise more and more rapidly, during expiration; if it were the only factor, the curve would slope upwards, more and more steeply, *i.e.* it would be concave upwards. (It may be objected that a constant pressure difference, as assumed, does not involve a constant  $\text{CO}_2$  influx, because as the air-sac shrinks the area of its walls, across which diffusion takes place, also diminishes; this, however, does not destroy the argument, because the volume must always diminish more rapidly than the area—therefore the increasingly upward trend of the curve remains.)

Such theoretical considerations, then, make it appear likely that two factors are principally responsible for the rise in alveolar  $\text{CO}_2$  during expiration, namely the continued diffusion of  $\text{CO}_2$  into the alveoli, and the progressive diminution in alveolar volume, the former tending to accelerate the rate of rise and the latter to retard it. How far the two factors, acting together, will counterbalance each other cannot be foretold on theoretical grounds, but from direct experimental observation of the alveolar  $\text{CO}_2$  curve in the latter two-thirds of moderately slow expiration during work, it seems that their combined effect is to make the alveolar  $\text{CO}_2$  rise at a uniform rate.

Provided the expiration is delivered at a uniform rate and not in a jerky or irregular fashion, these factors may be expected to act in a regular manner, and to counterbalance each other, not only in the part of expiration where the alveolar  $\text{CO}_2$  curve has been obtained directly, but throughout the whole of expiration. Hence it was assumed that the alveolar  $\text{CO}_2$  curve for the earlier part of expiration could be reconstructed by a backward prolongation of the straight part of the experimental curve (Fig. 7). A similar procedure also can be applied to the right-hand end of the experimental curve. The point *C* represents the last of the air that left the alveoli and also succeeded in reaching the small bags of the apparatus. A further quantity of air left the alveoli, but failed to reach the small bags; at the end of expiration it occupied the dead space. This further quantity can be represented by prolonging the experimental curve to the right over a distance equal to the dead space as already determined; this is done in Fig. 7 by the dotted line *CE*. The whole line *DE* now represents the  $\text{CO}_2$  concentration in air leaving the alveoli, and therefore in alveolar air, throughout expiration. In the figure, the line *DE* has been re-drawn as *D'E'*, with *D'* above zero on the abscissa: it can then be said that while air entering the small bags of the apparatus is changing its  $\text{CO}_2$  concentration in the way represented by the experimental curve *OABC*, the air in the alveoli is doing so along the line *D'E'*.



time of this breath, the subject, who had been giving Haldane-Priestley samples very shortly before, was not in respiratory and circulatory equilibrium. However that may be, it seems clear from our more extensive application of a method similar to that of Krogh and Lindhard, that the dead space in breathing during moderate exercise is of the order of 300 c.c. to 400 c.c. when the tidal air is between 2 litres and  $3\frac{1}{2}$  litres. While these results differ quantitatively from Haldane's, they do not conflict with his picture of what happens in atria and air-spaces during deep breathing, and they support his contention that the dead space should be regarded as a convenient physiological conception rather than a specified anatomical space.

### III. *The curve of alveolar $\text{CO}_2$ concentration during expiration.*

So long as  $\text{CO}_2$  is passing from the blood in the lung capillaries into the alveolar air, the alveolar  $\text{CO}_2$  concentration during expiration must rise, but it is not easy to analyse accurately the factors that determine the rate at which that rise will occur. There seem to be two quantities, variations in which are of the greatest importance; one of them changes in such a way as to favour a decreasing rate of rise, the other an increasing rate. They are:

1. The difference in  $\text{CO}_2$  pressure between the blood in the capillaries of the lung and the alveolar air. This is the force which makes  $\text{CO}_2$  diffuse across the endothelium and the alveolar wall. During expiration this force is diminishing, because

- (a) the  $\text{CO}_2$  pressure in the alveoli is rising while that in the blood is steady, or falling;
- (b) the oxygen pressure in the alveoli is falling; hence hæmoglobin is being less quickly oxidised, and is less effective in keeping the  $\text{CO}_2$  pressure of the blood high by displacing  $\text{CO}_2$  from combination with alkali.

This diminishing pressure difference means a diminishing influx of  $\text{CO}_2$  into the alveoli. This factor acting alone (assuming for the moment a constant alveolar volume) tends to cause the alveolar  $\text{CO}_2$  to rise, but to rise less and less rapidly, during expiration; if it were the only factor the curve of alveolar  $\text{CO}_2$  concentration would slope upwards, but less and less steeply, i.e. it would be concave downwards.

2. The volume of alveolar air (or, strictly, of air-sac air) into which a given amount of  $\text{CO}_2$  diffuses. As this diminishes, the effect of the added  $\text{CO}_2$  in raising the  $\text{CO}_2$  concentration increases. This factor acting alone (assuming for the moment a constant pressure difference and a constant

plot the curve (in Fig. 9) showing the rate at which air was expired, it was assumed that each bag ceased to fill with air at the moment when, as shown by the record, its successor opened; this may involve a slight error, but not more than two or three hundredths of a second. The curve indicates that, on the whole, the expiration was delivered at a uniform speed. There is, however, a small temporary hurry at the beginning, which appears only in some of the records, and may be instrumental; and there is a definite slowing down at the end of expiration, which is a feature of all records, and is a physiological phenomenon. In this kind of breathing, expiration slows off a little towards the end, and there may even be a slight pause before inspiration begins.

The time records therefore show that the assumption mentioned is in the main justifiable, but it is well to enquire how great may be the effect of the irregularities that do occur, especially on the mean value of the alveolar  $\text{CO}_2$  curve during expiration, which is to be discussed later on. The initial hurry is slight; it makes no difference to the flat part of the experimental curve, or to the slope of the alveolar air curve; it does make the dead space, as calculated, a few c.c. too large. The final slowing, however, needs further consideration. It means that if the experimental curve were plotted against *time* along the abscissa instead of against *volume*, it would be continued further to the right, and would bend downwards a little towards the end, thus becoming more nearly horizontal. Its average height would in that case be a little higher than that of the curve plotted against volume, and the same applies to the reconstructed alveolar air curve. At the same time, the prolongation to the right that represents the air in the dead space at the end of expiration would now be a continuation, not of the main slope of the curve, but of the more nearly horizontal part, and that would make the average height of the reconstructed alveolar air curve a little lower than it would otherwise have been. The average value, therefore, of the alveolar curve should not be seriously in error because of the slowing at the end of expiration, and the dead space calculation will not be affected by it.

#### IV. *The respiratory quotients of successive portions of an expired breath.*

The respiratory quotients of the successive portions of the breaths obtained in the experiments on the subject R.S.A. are given in Table III.

TABLE III. Respiratory quotients, Experiments 6 to 14.

Experi- ment no.	Bag 1	Bag 2	Bag 3	Bag 4	Bag 5	Bag 6	Whole divided breath	Expired air in tenth minute
6	1.77	1.03	1.01	1.00	1.01	—	1.02	1.03
7	1.60	1.12	1.11	1.09	1.08	—	1.11	1.10
8	1.26	1.08	1.10	1.09	1.07	—	1.09	1.09
9	1.28	1.06	1.06	1.07	1.05	1.03	1.06	1.03
10	1.19	1.00	1.03	1.01	1.01	.99	1.01	1.00
11	2.00	1.01	1.01	1.02	1.01	.99	1.01	1.00
12	1.90	.97	1.01	1.00	1.00	.97	.99	.98
13	2.23	1.26	1.32	1.27	1.25	1.24	1.28	1.19
14	1.12	1.04	1.09	1.07	1.06	1.04	1.06	1.05

In every case the first portion of the breath has an R.Q. much above the average for the whole breath. The remaining four or five portions have respiratory quotients that are close to the average; if anything, there is a slight tendency for the R.Q. to fall as the breath proceeds, and that of the last portion is always a little below the average.

In this example,  $D'E'$  rises from 4.5 p.c.  $\text{CO}_2$  at the beginning of a 3-litre expiration occupying 1.77 seconds, to 6.02 p.c.  $\text{CO}_2$  at the end, a rise of 1.52 p.c. or .51 p.c. per litre. In most of the other curves, the slope is similar. In the nine experiments on the subject R.S.A. it varies from .46 p.c.  $\text{CO}_2$  to .59 p.c.  $\text{CO}_2$  per litre of air expired, and it is not appreciably different in the shorter breaths from what it is in the longer ones. In moderate work, therefore, under the conditions of these experiments, the fluctuation of alveolar  $\text{CO}_2$  concentration throughout the respiratory cycle is of the order of .5 p.c.  $\text{CO}_2$  per litre of tidal air.

In the last paragraph but one, it was stipulated that the expiration be delivered at a uniform rate; the argument in that paragraph, indeed the whole reconstruction of the alveolar  $\text{CO}_2$  curve, rests on the assumption that it was delivered uniformly, and it was in order to justify this assumption that the records of the times of opening and closing of the valves were taken on the revolving drum. Those obtained in Exp. 12, which was a typical one, are shown in Fig. 9. The straight lines in the lower part of the figure indicate

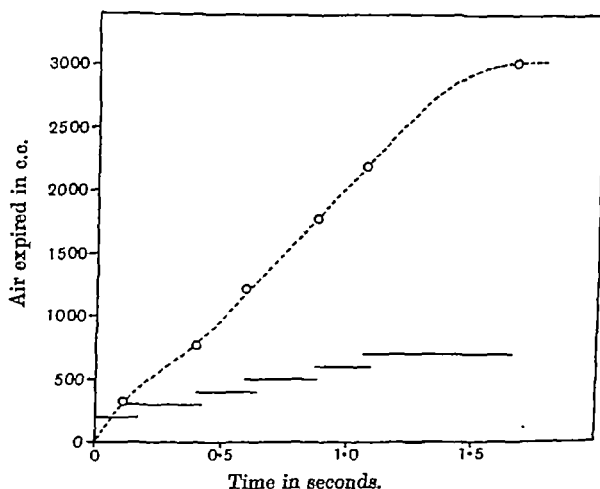


Fig. 9. Diagram showing the rate at which the divided breath was expired (Exp. 12).

The straight lines indicate the times during which the six valves were open. They correspond to Bags 1 to 6, in order from left to right.

The curve is obtained by plotting the volume of air already collected when each bag opens, against the time that has elapsed since the expiration began. The highest point corresponds to the closure of the sixth bag, very shortly after which the next inspiration began.

the time during which each bag was open, and are drawn from the measurements made on the tracing. They show a certain amount of overlap between adjacent bags, e.g. Bag 2 remained open for several hundredths of a second after Bag 3 opened, but this was inevitable, for had the overlap been reduced too far a stage would have been reached at which the expired air would have encountered a series of brief obstructions. In order to

alveolar air does not prove to have the same R.Q. as the expired air for the same period, then the method of sampling of alveolar air is at fault; these considerations make it clear that Pearce's criterion is unsound.

This phenomenon also emphasises the difference between the anatomical dead space and the physiological, functional or effective dead space. The diffusion of  $\text{CO}_2$  referred to above takes place into the anatomical dead space, which is the actual space in the respiratory passages from the mouth down to some undefined boundary that separates it from alveolar air. The physiological dead space is a conception involving the actual volume of the respiratory passages, the rate and volume of ventilation, the rate of diffusion of oxygen and  $\text{CO}_2$ , and various other factors. It is best defined as that volume of atmospheric air which must be added to the air that leaves the alveoli to make its concentration of oxygen or of  $\text{CO}_2$  the same as that of the expired air. On account of the diffusion of  $\text{CO}_2$  into the respiratory passages, the physiological dead space for  $\text{CO}_2$  must always be smaller than that for oxygen.

#### *V. Automatic sampling of alveolar air during exercise; average alveolar air.*

Several automatic devices have been introduced for the purpose of collecting alveolar air by withdrawing the last few c.c.'s of successive breaths throughout an experiment. Yandell Henderson<sup>(5)</sup> used one such device in his ethyl iodide method of determining the circulation rate, and considered the alveolar air so obtained to be equivalent to average alveolar air. Later Clark-Kennedy and Owen<sup>(1)</sup> published records of "alveolar" air obtained during exercise by a modified sampler, operated electrically; they did not consider that their sample had the composition of average alveolar air, but they did claim that its  $\text{CO}_2$  concentration could not be lower, nor its oxygen concentration higher, than those of average alveolar air.

The curve in Fig. 7 may be used to assess the value of these methods. Had one of them been used in this experiment, it would have given a sample containing about 5.75 p.c.  $\text{CO}_2$ , corresponding to the extreme end (C) of the experimental curve. The mean value for this whole curve is 5.26 p.c., so in this case the automatic sample would have contained about .5 p.c. more  $\text{CO}_2$  than the average alveolar air during expiration. In most of the other experiments the discrepancy would be of the same order, because the curves have much the same slope; it would of course be smaller in the shorter curves.

[Clark-Kennedy and Owen's apparatus was actually used in Exps. 13

The respiratory quotients for whole breaths which are in the neighbourhood of 1.00 may be looked on as normal for the tenth minute of such work as was performed. Those which are higher are probably due to overbreathing, but since the respiratory quotients for the whole tenth minute in those cases were also high, the overbreathing was not confined to the divided breath, and a certain steady degree of overbreathing in the tenth minute does not in any way impair the value of the results for the purposes of this paper.

The value for the R.Q. in the first portion is subject to a large experimental error, because the  $\text{CO}_2$  concentration and the reduction in the oxygen concentration are both small; but this cannot account for the uniformly high figures obtained. They are explained by the fact that the air which is last inspired and first expired comes in contact with a large area of mucous membrane in the respiratory passages, and also, in all probability, with some alveoli in the respiratory bronchioles, alveolar ducts, etc., as distinct from the air-sacs proper. This air has a very low  $\text{CO}_2$  pressure; therefore  $\text{CO}_2$  diffuses into it from the mucous surfaces and alveoli with which it comes into contact very much faster than it would into alveolar air containing 4 or 5 p.c. of  $\text{CO}_2$ . On the other hand, while this air has a higher oxygen pressure than alveolar air, it does not lose its oxygen much more readily to the blood in those surfaces and alveoli, because above the alveolar oxygen pressure the oxygen dissociation curve of hæmoglobin is relatively flat. The net result is that its gain of  $\text{CO}_2$  is out of proportion to its loss of oxygen, and its R.Q. is therefore high. The remaining portions are not affected in this way; their respiratory quotients are fairly steady, and therefore close to the average value for the whole breath.

The existence of the initial high R.Q. arising in this way makes it clear that the R.Q. of alveolar air and the R.Q. of *mixed expired air* cannot be the same. Mixed expired air contains alveolar air and air from the dead space: if the latter has an R.Q. higher than the R.Q. of the mixture, then the former, the alveolar air, must have a lower R.Q. than the mixture. How much lower depends on the relative proportions of alveolar and dead space air, or in other words the ratio of dead space to tidal air. In deep breathing, the dead space is small compared with the tidal air; hence, as the figures in Table III show, the alveolar R.Q. is only slightly lower than the expired air R.Q. On the other hand, in quiet breathing at rest, the dead space is a much larger fraction of the tidal air, and the alveolar R.Q. may fall considerably below the expired air R.Q., as Haldane first pointed out. Pearce(6), however, argued that if the

2. The curve representing the varying concentration of  $\text{CO}_2$  in the successive portions of an expired breath in moderate muscular exercise is shown to have a typical shape, which is described and illustrated.

3. From this curve the varying concentration of  $\text{CO}_2$  in the alveolar air during expiration can be deduced graphically; the alveolar  $\text{CO}_2$  rises steadily during expiration at the rate of about .5 p.c. per litre of air expired.

4. From this curve also the size of the physiological dead space of the subject can be deduced; it is found to lie between 300 c.c. and 400 c.c., increasing slightly with increase in the volume of the tidal air.

5. In this type of breathing, a sample obtained from the last air expired by Yandell Henderson's method of sampling alveolar air (or by similar methods), will have a  $\text{CO}_2$  concentration at least 10 p.c. higher than the average concentration of  $\text{CO}_2$  in the alveolar air throughout the respiratory cycle.

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4. Haldane. *Respiration*. Yale University Press, pp. 36 et seq. 1922.
5. Henderson and Haggard. *Amer. Journ. Physiol.* 73. p. 193. 1925.
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7. Krogh and Lindhard. *Ibid.* p. 431.
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and 14, and the samples obtained contained 5.28 and 5.35 p.c.  $\text{CO}_2$  respectively; the corresponding values read off from the curves were 5.54 and 5.43 p.c. The curves of course represent single breaths while the samples represent the mean of 18 breaths, and cannot be expected to agree exactly with the curves.]

It remains uncertain whether the average  $\text{CO}_2$  concentration of the alveolar air during expiration is the same as the average concentration throughout the whole respiratory cycle. Direct evidence seems unobtainable; theoretical considerations rest largely on assumptions. At first sight it appears that in inspiration, after a slight delay due to the re-inspiration of  $\text{CO}_2$ -laden air from the dead space, the alveolar  $\text{CO}_2$  concentration will fall at a steady rate, because it is influenced by the same factors as co-operated to produce the steady expiratory rise, only acting in the opposite direction. But there is one important difference; in expiration the alveolar volume is diminished by abstraction of air of the same composition as that left behind; in inspiration, the alveolar volume is increased by introduction, not of air similar in composition to that already there, but of fresh air containing no  $\text{CO}_2$ . This speeds up the fall in  $\text{CO}_2$  concentration in the first half of inspiration, so that in all probability the average alveolar  $\text{CO}_2$  concentration during inspiration is lower than that during expiration. If that be so, the average alveolar  $\text{CO}_2$  concentration for the whole cycle is also lower than the average during expiration, and the error involved in regarding an automatic sample as average alveolar air is greater than that already indicated. It is safe to conclude that, in this type of breathing, the error involved by regarding the  $\text{CO}_2$  concentration of an automatically collected sample as that of average alveolar air is at least + 10 p.c.; this error will re-appear in any calculation of circulation rate (such as Yandell Henderson's) which so regards the automatic sample, and will probably be as great for ethyl iodide as it is for  $\text{CO}_2$ .

(For a similar reason it is clearly unsafe to assume that the  $\text{CO}_2$  given off into the alveoli during expiration is half of that given off during the whole cycle; had that assumption been permissible it would have been possible from the data in Fig. 7 and Table I to calculate the subject's residual and reserve air.)

#### SUMMARY.

1. An apparatus has been devised for collecting a single expired breath from a human subject during moderate muscular exercise, and dividing it into six separate successive portions suitable for measurement and analysis.

after which blood was drawn from the femoral artery. Approximately 50 c.c. were taken into a paraffined syringe containing sufficient potassium oxalate and sodium fluoride to give a concentration of 0.4 p.c. of the former and 0.1 p.c. of the latter. After mixing, the blood was kept under paraffin until required. In the earlier observations ice was employed as a further check on any spontaneous change, but during the acidosis trouble was experienced from hæmolysis and to obviate this the chilling was omitted.

The oxygen content of the blood and its capacity were determined with the Haldane blood gas apparatus (4), its  $\text{CO}_2$  content by the constant volume apparatus of Van Slyke and Neill (5) and the  $\text{pH}$  by the colorimetric method of Hastings and Sendroy (6). Three or four samples were equilibrated with different pressures of  $\text{CO}_2$  by the method described in an earlier communication (7). After this the samples were examined for their  $\text{CO}_2$  content and  $\text{pH}$ , and the exact  $\text{CO}_2$  tension to which they had been exposed in the tonometers was determined. A slightly larger sample was placed in the tonometer calculated to contain the tension of  $\text{CO}_2$  nearest to that of the alveolar air; the plasma was separated from the portion of this sample remaining after the above determinations had been completed, and its  $\text{CO}_2$  content was also determined. From the plasma  $\text{CO}_2$  and the  $\text{CO}_2$  tension to which it had been exposed the true  $\text{pH}$  of this sample was calculated using 6.10 as  $\text{pK}$  (8) and 0.541 as the solubility coefficient of  $\text{CO}_2$  in plasma (9). This figure was compared with the observed colorimetric  $\text{pH}$  and a correction was obtained which was applied to the colorimetric reading of the  $\text{pH}$  of the arterial blood to obtain its true  $\text{pH}$  (10). On three occasions the  $\text{CO}_2$  in the plasma of the arterial blood was determined, and from this and the arterial  $\text{CO}_2$  tension a second figure for  $\text{pH}$  was obtained by calculation.

Curves were constructed connecting (i) the  $\text{CO}_2$  tension and  $\text{CO}_2$  content of the samples, and (ii) the  $\text{CO}_2$  tension and crude colorimetric  $\text{pH}$ . By placing the points for the  $\text{CO}_2$  content and uncorrected  $\text{pH}$  of the arterial blood on these curves two figures were found for the tension of  $\text{CO}_2$  in the arterial blood (Chart I). As is shown by Table I these figures agreed closely, the greatest difference being 2.3 mm.

Two observations were made of the normal state on February 7th and 16th. The main experiment was begun on March 27th; on this day the subject took 20 gm. of ammonium chloride with a large quantity of water. On the following morning he showed definite clinical evidence of poisoning; he was short of breath on the least exertion and was less



## THE ARTERIAL BLOOD IN AMMONIUM CHLORIDE ACIDOSIS.

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IN 1921 one of us, J. B. S. H.<sup>(1)</sup>, showed that an acidosis could be produced in man by taking large amounts of ammonium chloride by the mouth. It was found to cause extreme "air hunger" and reduction of the capacity for physical effort, reduction of the alveolar  $\text{CO}_2$  and the  $\text{CO}_2$  capacity of the blood, and an increase in the excretion of acid, ammonia and phosphate in the urine. These observations indicated the existence of an acidosis but did not permit a complete estimate to be made of the nature and extent of the changes which had taken place in the acid-base equilibrium of the blood. A great diuresis was noted and the relation of this to the acidosis as effect to cause was supported by subsequent experimental work by Haldane, Hill and Luck<sup>(2)</sup>. Since then this substance and salts with a similar physiological action, such as calcium chloride and ammonium nitrate, have achieved a definite place among diuretics of clinical value in the treatment of oedema and as corrective agents in the treatment of alkalosis and tetany. The present paper contains an account of direct investigations into the changes which occurred in the arterial blood of the same subject during a similar period of ammonium chloride intoxication.

*Experimental.* The ammonium chloride was taken and the observations on the acidosis were made in March 1927, but the control observations on the arterial blood in its natural state were made as opportunity offered between February and October of that year. For these controls the subject came to the laboratory in the morning without breakfast but for the period of acidosis he was kept in the hospital.

The routine of the observations was as follows. After a short rest in the laboratory the subject determined his own alveolar  $\text{CO}_2$  with the Haldane gas analysis apparatus<sup>(3)</sup>, taking the average of inspiratory and expiratory samples. He then lay down on a bed for at least half an hour

equable than usual. On March 28th he took 25 grm. during the course of the day and on the following day 15 grm. Blood was drawn each morning before any of the day's ammonium chloride was taken and also on the morning of the 30th. Two observations were made afterwards, one in May and one in October.

*Results.* The results are recorded in Table I and in Charts I, II and III. With regard to the figures in general it may be noted that the figures for  $pH$  obtained by the two methods agree very closely. The  $CO_2$  tension of the arterial blood derived from the  $pH$  curve agrees well with that derived from the  $CO_2$  content curve. The  $CO_2$  tension of the arterial blood was 3 to 5 mm. greater than that of the average alveolar air, except on the last occasion when the subject had inadvertently taken his breakfast and there was a small difference in the reverse direction.

*Preliminary observations.* The two preliminary observations show a small amount of variation in the level of the  $CO_2$  tension-volume and  $CO_2$  tension- $pH$  curves. The arterial  $pH$  was the same. The  $CO_2$  tension in the alveolar air and arterial blood had fallen on the second occasion by 3 mm. and the  $CO_2$  content of the arterial blood had fallen by  $\frac{1}{2}$  volumes per cent.

*Period of acidosis.* By the morning of the second day, after 20 grm. of ammonium chloride had been taken the day before, considerable changes had occurred in the blood. The  $CO_2$  content of the arterial blood, its  $pH$  and its  $CO_2$  tension had all fallen to a corresponding extent. There was evidence of concentration of the blood in the increase of the oxygen capacity. The  $pH$  and  $CO_2$  curves were at a much lower level for any given  $CO_2$  tension than before.

On the morning of the third day after another 25 grm. of ammonium chloride had been taken all these changes were more definite. The concentration of the blood was more obvious. The  $CO_2$  tension had been reduced to almost half that in the control period and in spite of this the  $pH$  had fallen yet lower.

The result of the 15 grm. taken on this day was shown by a yet greater concentration of the blood on the morning of the fourth day, the oxygen capacity rising to 22.8 volumes per cent. The  $CO_2$  tension remained unchanged but a continued fall in the  $CO_2$  content involved a further fall in  $pH$ .

It is evident that from the first there was a comprehensive disturbance of all the equilibria involved in the acid-base relationship of the blood. No single factor was held constant at the expense of the others. The change in the  $pH$  of the plasma at the first observation in the period

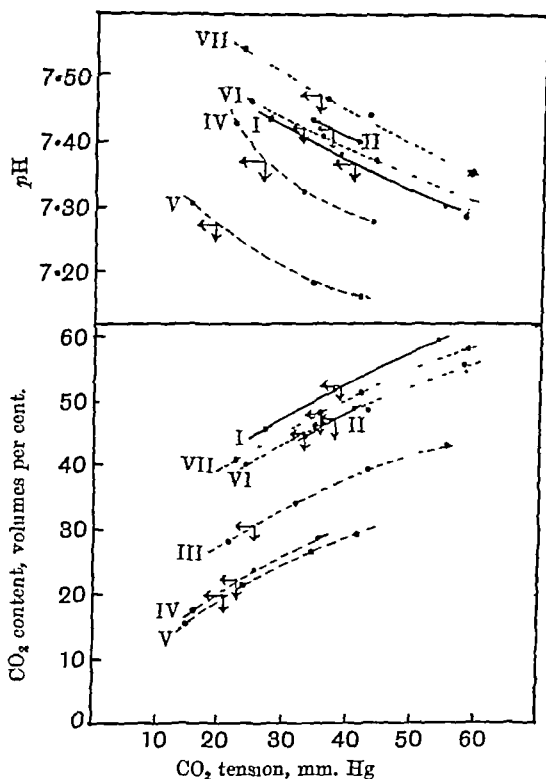


Chart I. The curves are numbered to correspond with the observations as recorded in Table I. Curves I and II were obtained before the acidosis; III, IV and V during it; and VI and VII after it. The arrows are drawn from the arterial points.

TABLE I.

Exp	Date	Arterial blood										Remarks	
		Oxygen		pH		Carbon dioxide							
		Capa- city Vol p.c.	Satura- tion p.c.	Colori- metric. (Cor- rected)	Calcu- lated	Con- tent Vol. p.c.	Tension		CO <sub>2</sub> con- tent. CO <sub>2</sub> curve. mm.	Alveo- lar air Carbon dioxide tension mm.			
							pH- CO <sub>2</sub> tension curve mm.	CO <sub>2</sub> tension curve. mm.					
I	7. ii 27	18.3	94	7.40	—	52.8	40.4	38.8	36.1				
II	16. ii. 27	—	—	7.42	—	47.9	36.5	38.8	33.4				
III	28. iii. 27	18.85	95	7.36	—	31.0	25.6	25.0	24.5	20 grm. NH <sub>4</sub> Cl taken on March 27th			
IV	29. iii. 27	20.8	97	7.31	7.31	22.85	—	22.5	18.3	25 grm. NH <sub>4</sub> Cl taken on March 28th			
V	30. iii. 27	22.8	97	7.29	7.29	20.25	18.5	20.5	18.3	15 grm. NH <sub>4</sub> Cl taken on March 29th			
	31. iii 27	—	—	—	—	—	—	—	25.2				
VI	12. v. 27	15.7	96	7.43	7.44	45.7	31.8	33.2	30.5				
VII	29. x. 27	16.8	96.5	7.45	—	48.9	34.0	35.0	36.6				

equable than usual. On March 28th he took 25 gm. during the course of the day and on the following day 15 gm. Blood was drawn each morning before any of the day's ammonium chloride was taken and also on the morning of the 30th. Two observations were made afterwards, one in May and one in October.

*Results.* The results are recorded in Table I and in Charts I, II and III. With regard to the figures in general it may be noted that the figures for  $pH$  obtained by the two methods agree very closely. The  $CO_2$  tension of the arterial blood derived from the  $pH$  curve agrees well with that derived from the  $CO_2$  content curve. The  $CO_2$  tension of the arterial blood was 3 to 5 mm. greater than that of the average alveolar air, except on the last occasion when the subject had inadvertently taken his breakfast and there was a small difference in the reverse direction.

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It is evident that from the first there was a comprehensive disturbance of all the equilibria involved in the acid-base relationship of the blood. No single factor was held constant at the expense of the others. The change in the  $pH$  of the plasma at the first observation in the period

of acidosis did not take the  $pH$  outside the normal limits; this is shown by point III in Chart II in which the normal area is taken from Fig. 1 of Hastings, Neill, Morgan and Binger(11). This chart also shows that

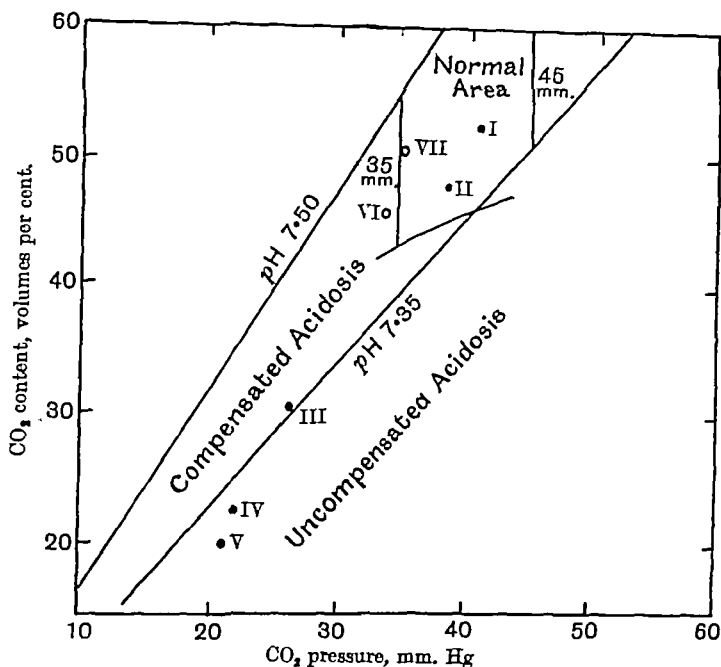


Chart II. The arterial points are numbered to correspond with the observations in Table I.

the arterial point moved in the same direction from the beginning of the acidosis, and that the "uncompensated acidosis" of the two later observations—points IV and V—was merely a progression from and accentuation of changes already apparent in the "compensated acidosis" of the earlier observation—point III. In comparable acidoses Koehler(12) found that the pressure of  $CO_2$  in venous blood was relatively constant and that the  $pH$  fell to much lower figures than we found in the arterial blood in this experiment; in fact by the increased respiratory activity of our subject the fall in  $pH$  was limited to about half that which would otherwise have occurred.

*Subsequent observations.* Six weeks later it appeared from the examination that the normal state had not been regained. The alveolar  $CO_2$ , the arterial  $CO_2$  tension and content were all lower than in the preliminary period although in other similar experiments the alveolar  $CO_2$  had

returned to normal in a few days. The oxygen capacity was definitely reduced. Seven months later recovery appeared complete in regard to acid-base balance but the oxygen capacity was still slightly low.

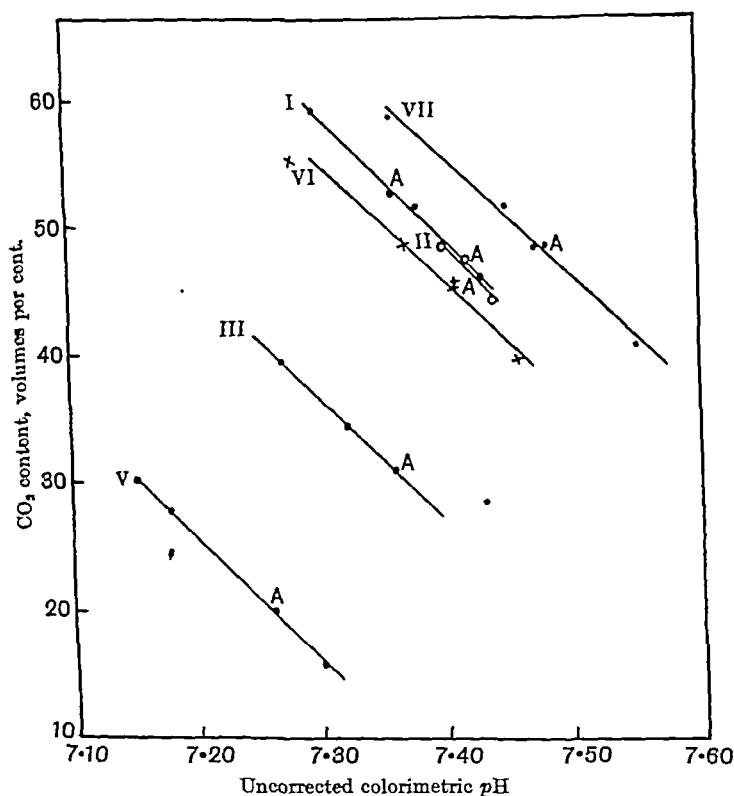


Chart III. The lines are numbered to correspond with the observations in Table I. The points marked *A* are the arterial points.

*Buffer capacity.* Chart III shows the *pH* and  $\text{CO}_2$  content of the arterial and equilibrated specimens of the blood obtained on the different occasions. Straight lines have been drawn through the series of points obtained on each day. The change in level of these lines indicates that a great decrease of alkali available for carrying  $\text{CO}_2$  took place during the acidosis, but the fact that their slope remains approximately the same shows that no great change in buffering occurred. The amounts of  $\text{CO}_2$  required to change the *pH* by 0.10 were for the preliminary days (I and II) 10.5 and 10.0 volumes per cent., for the second and fourth days of the acidosis (III and V) 10.8 and 9.8, and for the subsequent

examinations (VI and VII) 9.5 and 9.7. Charts prepared by the method described by Barcroft<sup>(13)</sup> did not give any additional information.

The change in buffering between the first two examinations and the last two may be explained by the lower concentration of hæmoglobin found in the latter. By the same reasoning it would be expected that the considerable increase in hæmoglobin which occurred during the acidosis would be reflected in a very definite increase in buffering power. This was not the case. There are two other factors to be considered.

(a) Changes in the buffers other than hæmoglobin. The figures show that the bicarbonate was reduced to two-fifths of its customary amount. We did not obtain figures for the inorganic phosphate, but Haldane, Wigglesworth and Woodrow<sup>(14)</sup> have shown that after similar doses of ammonium chloride on another occasion the inorganic phosphate in the blood of J. B. S. H. was diminished. The plasma protein probably increased in concentration with the hæmoglobin and would therefore tend to have increased the buffering. According to Van Slyke, Hastings and Neill<sup>(15)</sup> oxyhæmoglobin constitutes 78 p.c. of the total buffering of horse blood, bicarbonate 7.4 p.c. and other buffers 14.6 p.c. The decrease of bicarbonate to less than half the normal amount, which we actually found, could not mask the effect of an increase of hæmoglobin by over one-fifth; and since the other buffers referred to above consist in large part of the plasma proteins, the concentration of which was probably increased, there is little possibility of explaining the constancy of the buffering in this way.

(b) From Chart III it may be seen that the points from which line V was drawn were much further to the left, i.e. more acid than those from which the other lines were drawn; this corresponded to an actual change in the reaction of the circulating arterial blood in the same direction and of similar degree. By this approach to its isoelectric point it seems possible that the buffering of the hæmoglobin may be rendered less efficient, but in practice Van Slyke, Hastings, Heidelburger and Neill<sup>(16)</sup> did not find any change in the buffer effect of horse hæmoglobin between pH 7.2 and 7.5.

We have not therefore been able to explain the changes in the buffering of the blood at the height of the acidosis by the known behaviour of animal blood *in vitro*, but this is perhaps not surprising considering the complexity of the factors concerned.

*Hæmatology.* Haldane, Hill and Luck<sup>(2)</sup>, in their study of calcium chloride acidosis, found that the total number of white cells was increased by 30 p.c. and that there was an increase in the number of lymphocytes.

Differential blood counts were made by Dr A. E. Carmichael on this occasion during the course of the acidosis. His results are presented in Table II. The blood was taken by finger prick in the forenoon while J. B. S. H. was fasting.

TABLE II. Blood counts.

Exp.	Date	Red cells	Hæmo- globin (Haldane)	White cells	Polymorph	Lymphocytes	Large hyaline	Remarks
II	16. ii. 27	4,080,000	80	6000	3210	2160	150	
III	28. iii. 27	4,540,000	94	9000	3690	4300	450	20 grm. $\text{NH}_4\text{Cl}$ taken on March 27th
IV	29. iii. 27	5,340,000	111	7800	3744	2700	624	25 grm. $\text{NH}_4\text{Cl}$ taken on March 28th
	After bleeding	5,100,000	109	6800	2822	2100	986	
V	30. iii. 27	5,680,000	117	6200	3162	1302	1302	15 grm. $\text{NH}_4\text{Cl}$ taken on March 29th
	31. iii. 27	5,220,000	108	6400	2498	2369	1152	
VII	29. x. 27	5,230,000	96	7400	4218	2664	333	

The count of the red cells followed the change in the concentration of the hæmoglobin. The ammonium chloride or else the acidosis appeared to have a deleterious effect on the erythrocytes, increasing their fragility, for as the acidosis developed it became increasingly difficult to avoid causing hæmolysis in the experimental manipulations; the observations on the third day of the acidosis were spoiled by the occurrence of hæmolysis which rendered the reading of the pH tubes impossible. The same effect was observed in an unpublished experiment on J. B. S. H. The low figure found for the hæmoglobin six weeks later may possibly be connected with the same damage to the cells shortening their lives, but the time is so long and the change in hæmoglobin so small that the evidence does not warrant a conclusion.

The white cells showed considerable but irregular changes in number. The lymphocytes were the more numerous at the beginning of the acidosis than before but their number fell steadily throughout its course. The large hyaline cells increased steadily from 150 per cubic millimetre to 1302 at the height of the acidosis and began to decrease as soon as the recovery from the acidosis commenced. This was the most striking change in the leucocyte picture and had been noted by C. S. Hicks in unpublished work on the same subject. An increase of these cells apart from a general leucocytosis is known to occur in typhoid fever, malaria and other infections<sup>(17)</sup>. It is a regular and striking feature of a fatal disease of rabbits due to infection with a specific organism which has been named *Bacterium monocytogenes*<sup>(18)</sup>; "infectious mononucleosis"<sup>(19)</sup> is possibly a human disease of similar nature. Changes in



these cells have not been observed in the acidosis of diabetes or nephritis, and no drug is known to have any effect on them. These cells are derived from the reticular and endothelial systems(17) so it appears possible that ammonium chloride has a toxic action on the red cells increasing their fragility and on the endothelial cells giving rise to this monocytic response.

#### SUMMARY AND CONCLUSIONS.

A state of acidosis was produced in 24 hours by taking large doses of ammonium chloride by the mouth, and was intensified by continuing this process for two more days.

Examination of the arterial blood showed that after 24 hours all the equilibria of acid-base regulation were disturbed. The  $\text{CO}_2$  content and the  $\text{CO}_2$  tension of the blood were less, the reaction more acid, and the raised content of hæmoglobin indicated that concentration of the blood had occurred. None of these factors had been held constant at the expense of the remainder, but by changes in them all a new equilibrium had been established. All these changes became more pronounced as the ammonium chloride intoxication was continued and the acidosis became more severe.

$\text{CO}_2$  tension- $\text{CO}_2$  content and  $\text{CO}_2$  tension-pH curves showed that for a given tension of  $\text{CO}_2$  the blood absorbed less  $\text{CO}_2$  and became more acid as the acidosis progressed.

The buffering of the blood remained unchanged in spite of the increase in hæmoglobin concentration.

The acidosis was accompanied by an increase in the number of large hyaline leucocytes, and by an increased tendency of the red cells to hæmolyse. These effects and a mild grade of subsequent anæmia suggested that the ammonium chloride had a damaging effect on the blood and endothelial cells.

We wish to express our thanks to the Medical Research Council for personal grants to two of us (R. H. and G. C. L.).

## REFERENCES.

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## THE CONDITIONS GOVERNING THE BLOOD CAPACITY OF THE LUNGS.

BY I. DE BURGH DALY.

*(From the Physiology Institute, Cardiff and the Physiology Department,  
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A CONSIDERABLE amount of evidence has accumulated in favour of the view that expansion of the lungs by a negative pressure increases their blood volume(1, 2, 3, 4, 5). An analysis of the mechanism responsible for this augmented blood capacity has been given in a paper by de Jager(2), who enumerates the important factors as (a) the pressure inside the blood vessels, (b) the difference in pressure between the intrathoracic space and the intrapulmonary space, (c) the blood-pressure fall throughout the lungs, and (d) changes in the elastic properties and in the calibre of the vessels produced by lung expansion. Under the last heading it may be stated that expansion of the lungs is said to stretch the smaller blood vessels both longitudinally and transversely, but opinions differ as to whether the longitudinal force diminishes the calibre more than the transverse force increases it (Zuntz(5), Cloetta(6)). From the mechanical standpoint the principles laid down by de Jager must govern the blood capacity of the lungs under negative pressure ventilation, but work in the past has been concentrated chiefly upon correlating the changes in arterial and in venous pulmonary pressures with those in the blood capacity. Little attention has been paid to the relation between blood flow and blood capacity. In this connection, Barcroft(7), writing on the conditions at high altitudes, points out the gain to the system of an increased vascular bed. The gain would be that, "at the low oxygen pressure prevalent at such heights, the oxygen would have more time to diffuse into blood if the minute volume were not increased, or alternatively if the minute volume were increased, the oxygen exchange required of each cubic centimetre of blood would be reduced so that less oxygen need pass through the wall of any one capillary." M. Krogh(8), however, failed to find definite evidence of alterations in diffusion as a result of such changes in blood flow which occur readily during rest.

It appears desirable therefore to obtain some information with regard

to the relation between blood flow and capacity of the pulmonary vascular bed, the more so as the experiments of Cohnheim and Litten<sup>(9)</sup> and of Shaw Dunn<sup>(10)</sup> suggest that under certain conditions the extent to which parts of the vascular area are opened up may vary. If this is so, measurement of blood-pressure values in the lungs may not give such valuable information with regard to the state of the vascular bed as measurements of blood flow, for the sudden opening up of a vascular area may only give rise to a transient change in pulmonary arterial pressure (Tigerstedt<sup>(11)</sup>).

The pulmonary circulation in the heart-lung preparation has been studied by Fühner and Starling<sup>(12)</sup>, Yas Kuno<sup>(13)</sup>, Straub<sup>(14)</sup> and by Anrep and Bulatao<sup>(15)</sup>. These investigators used positive pressure intratracheal ventilation but no measurements of the blood capacity of the lungs were made. As is well known, positive pressure ventilation produces widely different results on the blood capacity of the lungs from those produced by negative pressure ventilation; moreover, the former in the heart-lung preparation only indirectly influences the output of the heart, whereas the latter if the whole preparation is placed under negative pressure (Daly<sup>(16)</sup>) directly governs the output of the heart. It follows that with negative pressure ventilation the respiratory pressure<sup>1</sup> will determine the blood capacity of the lungs by virtue of its effect on the diastolic filling of the heart and also by its effect on lung expansion. The flow, under these conditions, is limited by the response of the heart to the respiratory pressure fall and, owing to the absence of nervous and additional mechanical influences which generally come into play during increased pulmonary ventilation, will not be so large as that which might occur in the whole animal. For this reason and because it was desired to separate the influence of lung expansion from that of blood flow on the capacity of the lungs, two series of experiments have been carried out, the first on the heart-lung preparation with negative pressure ventilation and the second on the isolated perfused lungs in which the blood flow is varied independently of the respiratory pressure. The total blood capacity variations have been measured, but no information has been obtained with regard to the rôle played by the capillaries.

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## I. EXPERIMENTS ON THE HEART-LUNG PREPARATION.

In all experiments dogs have been used fully anaesthetised with chloralose 0.1 grm. per kilo body weight injected intravenously. The heart and lungs have been set up in the closed circuit negative pressure ventilation apparatus with means provided for measuring the volume changes in the preparation as described in a previous paper (16).

*Results.* On gradually reducing the mean respiratory pressure, the difference between the inspiratory and expiratory pressures being kept

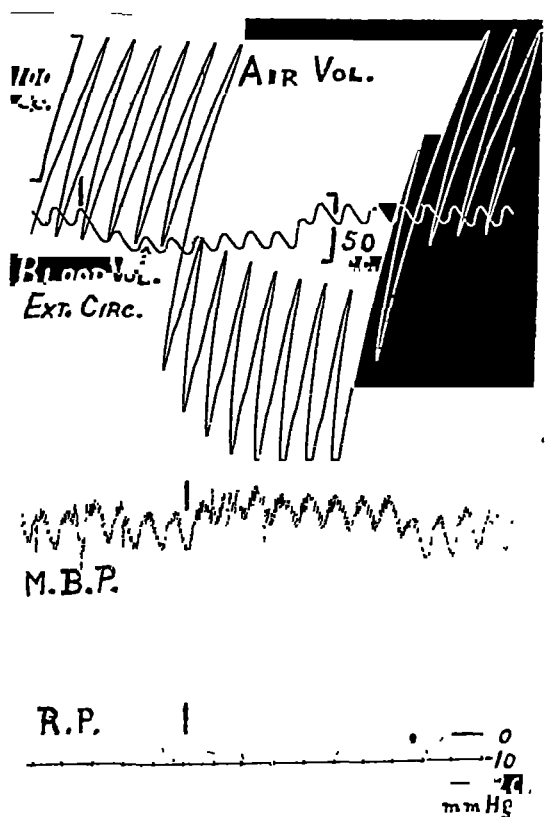


Fig. 1. Heart-lung preparation. Dog 9.0 kilos. Effect of reducing the mean respiratory pressure from  $-7$  to  $-9$  and back to  $-5$  mm. Hg. The peripheral output measured 500, 785 and 440 c.c. per minute respectively. R.P.=respiratory pressure. Air volume of the lungs taken with a spirometer. A diminution in the volume of the external circuit denotes an increase in the heart and lungs. Corresponding ordinates are marked by vertical lines.

constant, the blood volume of the heart and lungs is markedly increased, Figs. 1 and 2. The quantity of blood entering the preparation will depend

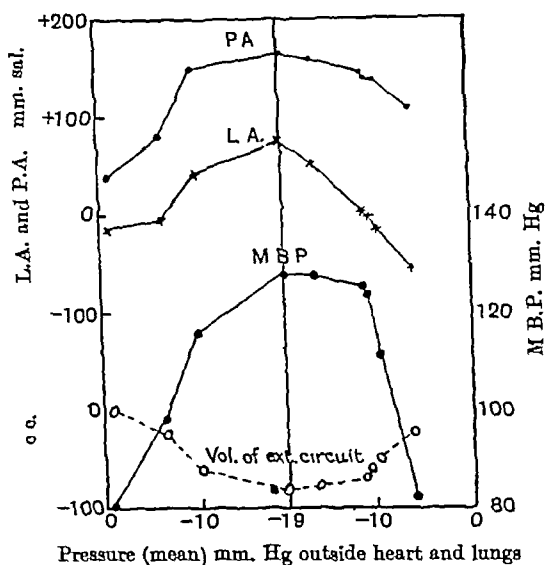


Fig. 2. Heart-lung preparation. Dog 8 kilos P.A.=pulmonary arterial pressure.  
L.A.=left auricular pressure.

in part on the size and elasticity of the lungs, so that large variations in blood volume changes in response to the same respiratory pressure fall might be expected from one preparation to another. This is evident from Table I in which ten experiments are collected. For the purpose of comparing the experiments, the percentage rise in peripheral output and the actual increase in the blood volume in response to a fall of respiratory pressure of 1 mm. Hg is calculated in columns 6 and 7 respectively. With such variable results it is only possible to obtain an approximate estimate of the volume changes, but Exp. 3 shows it is possible for a 1 mm. Hg pressure fall to augment the peripheral flow by 7.8 p.c. and the blood volume by 7.0 c.c. The peripheral output response will depend in part on the functional capacity of the heart.

It will be seen from the protocols of Exp. 6, *a*, *b* and *c*, which are taken from the same preparation, that the blood entry to the heart and lungs for every mm. Hg fall in respiratory pressure, is very much larger with open than with intact pericardium which presumably is due to the greater volume changes in the heart. Examination of Exps. 8, 9 and 10, however, show that the blood entry even with an open

pericardium may not differ to any great extent from those experiments in which the pericardium is intact, if one experiment is compared with another.

TABLE I. Effect of reducing the respiratory pressure upon the output and blood volume of the heart-lung preparation.

Exp.	Dog weight kilos	M.R.P. mm. Hg	P.O. c.c. per minute	H.L. volume increase in c.c.	M.B.P. mm. Hg	per mm. Hg M.R.P. fall	
						P.O. increase p.c.	H.L. volume in c.c.
1	8.0	- 1.0 - 19.0	220 680	63	80 128	11.6	3.5
2	5.5	- 2.0 - 13.5	— —	14	80 102	—	1.2
3	7.0	- 1.0 - 6.0	430 600	35	110 120	7.8	7.0
4	6.5	+ 3.0 - 5.0	240 625	22	78 86	20.0	2.8
5	6.0	- 2.5 - 3.5	430 470	4	100 102	9.3	4.0
6	9.0	- 1.5 - 13.5	375 650	39	80 87	6.4	3.4
6 a	9.0	- 3.0 - 9.0	750 835	21	91 92	1.9	3.5
6 b	9.0	- 5.5 - 12.5	400 600	102	94 102	7.1	14.6
6 c	9.0	- 5.5 - 13.0	430 545	103	96 100	3.6	13.7
7	6.0	- 6.0 - 12.0	600 750	61	95 102	4.2	10.1
8	8.0	- 1.5 - 7.5	650 1000	22	99 104	9.4	3.8
9	7.5	- 2.5 - 9.0	365 600	19	85 93	9.9	2.9
10	7.0	- 3.5 - 14.5	165 500	20	84 95	18.5	1.8
Column	1	2	3	4	5	6	7

M.R.P.=mean respiratory pressure. P.O.=peripheral output. H.L.=heart-lung preparation. M.B.P.=mean blood-pressure. Pericardium closed in Exps. 1 to 6 a and open in Exps. 6 b to 10.

The immediate effect of opening the pericardium is to augment the blood volume of the preparation; in two experiments this increase amounted to 35 and 25 c.c. Opening of the pericardium leads to an increase in the peripheral output which, since this takes place even with the mean respiratory pressure remaining constant, depends upon the heart being enabled to do more work owing to an increase in its diastolic volume. The extra amount of blood in the heart is, however, not the

only factor determining the augmented blood volume of the preparation, some of the increase is due to the extra amount of blood in the lungs.

It may be concluded that the blood volume increase of the preparations in response to a respiratory pressure fall shows individual variations which may be very large, and if the pericardium is open, the heart plays an important part in determining the total volume changes.

## II. PERFUSION EXPERIMENTS ON THE ISOLATED LUNGS.

In all experiments dogs have been used. The animal is placed under chloroform and ether anæsthesia and then bled through one of the carotid arteries. The thorax is opened and cannulæ introduced into the pulmonary artery and the left auricular appendix. The lungs are washed through with defibrinated blood obtained from the same animal and set up in the respiratory chamber. Before the lungs are washed through, a clamp, Fig. 3 c, is placed on the auriculo-ventricular junction to prevent

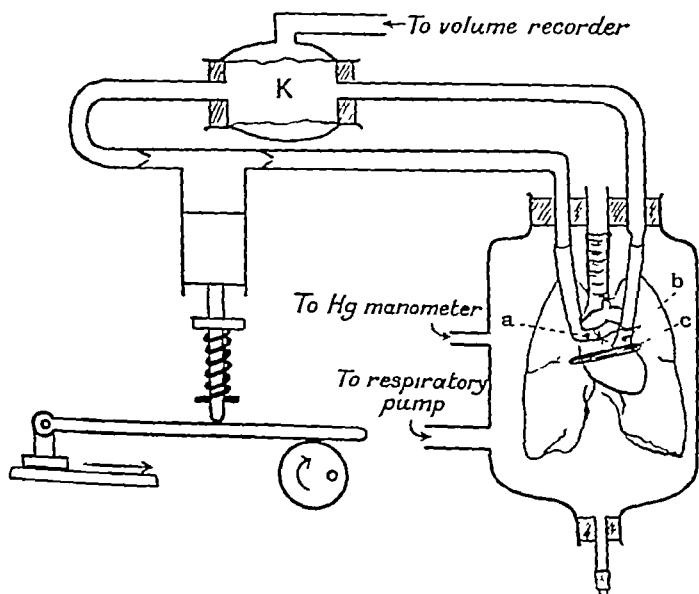


Fig. 3. Arrangement for perfusion of the isolated lungs. See text.

blood from passing from the left auricle to the ventricle. The clamp is so arranged as to diminish the size of the left auricle, the walls of which are kept on the stretch. This procedure is adopted to prevent capacity changes in the auricle taking place when the respiratory pressure is

lowered. Inspection of the auricle in the respiratory chamber showed little or no bulging on reducing the chamber pressure, and the stretched condition of its walls did not interfere with the free flow of blood from the pulmonary veins. The cannula inserted in the pulmonary artery is connected to the output side of the pump and the cannula in the left auricular appendix to one end of a rubber bag *K* (Fig. 3), the other end being joined to the input side of the pump. The whole system is filled with defibrinated blood and circulation through the lungs maintained by rotation of the pump. The rate of flow is measured with a Stolnicov and Pavlov stromuhr and volume changes in the lungs by recording the volume of the rubber bag.

The design of the perfusion pump demands further mention. Since blood capacity changes in the lungs are being measured, it is essential that no capacity changes should take place in the pump or in the connecting tubes when the pulmonary resistance varies. It is found that a rubber diaphragm pump as designed by Dale and Schuster (17) responds to an increase in output resistance by an appreciable dilatation which shows as a diminution in the rubber bag *K*. This difficulty has been overcome by using a large glass syringe as the pump, a tightly fitting piston being absolutely necessary otherwise a film of blood and lubricant causes binding between the piston and the cylinder. The syringe, selected from a batch of twelve, is a three-piece Record of 20 c.c. capacity. Variations in perfusion rate are brought about by the adjustment of the fulcrum of the lever actuating the piston, the pump is therefore a modification of the type used by Dale and Schuster to meet the special requirements already described. The output as measured with a stromuhr remains constant against resistances of 10 to 70 mm. Hg, the maximum output at 100 strokes per minute being 500 c.c. per minute. Larger flows are obtained by running a similar pump in parallel off the same shaft.

**Results.** The lungs are ventilated by rhythmic changes in the respiratory pressure, the mean pressure being lowered either by sucking air out of the chamber, in which case the difference between the inspiratory and the expiratory pressure remains constant (method I), or by lowering the inspiratory pressure, the expiratory being kept constant (method II).

TABLE II.

Perfused isolated lungs. Dog 11.0 kilos. Blood flow 450 c.c. per minute. Respiration rate 12 per minute. Mean respiratory pressure reduced by method II.

Respiratory pressure mm. Hg			Mean blood vol. of lungs	"Respiratory blood"
Inspiratory	Expiratory	Mean	c.c.	c.c.
-10.0	-2.5	- 8.25	<i>x</i>	14.0
-14.0	-2.5	- 8.25	<i>x</i> + 3	17.5
-17.0	-2.5	- 9.75	<i>x</i> + 5	20.0
-19.0	-2.5	-10.75	<i>x</i> + 7	22.5
-20.0	-2.5	-11.25	<i>x</i> + 9	23.0

Blood flow 625 c.c. per minute. Mean respiratory pressure reduced by method I. Other conditions the same.

-18.0	- 3.0	-10.5	<i>x</i>	18.5
-24.0	- 8.0	-16.0	<i>x</i> + 9	17.0
-26.0	-10.0	-18.0	<i>x</i> + 16	20.0

For producing the pressure changes by method II, a radial slot is cut in the arm actuating the crank of the respiratory pump and the movement produced is similar to that of the Dixon perfusion pump (18).

The results of two experiments are shown in Table II and in Fig. 4;

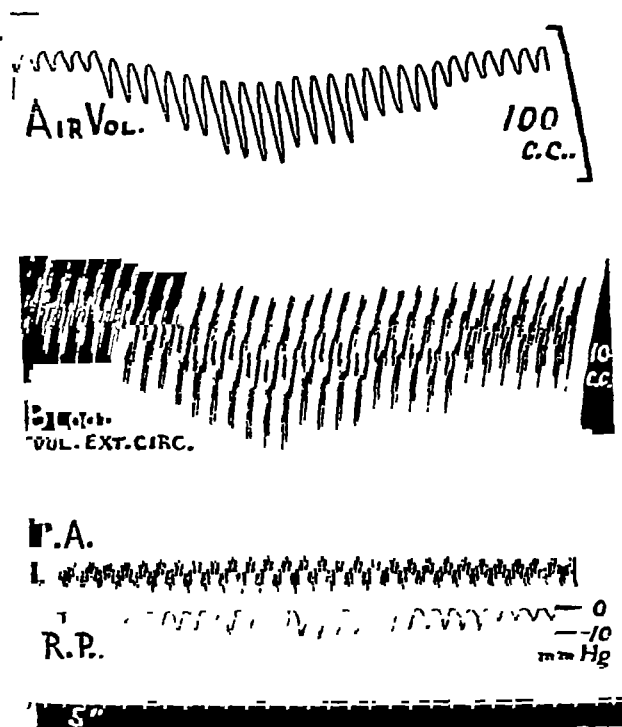


Fig. 4. Perfused lungs. Dog 6.0 kilos Effect of reduction in the mean respiratory pressure.

it is evident that the mean blood volume of the lungs is determined by the mean respiratory pressure, and the amount of blood entering and leaving the lungs at each respiratory cycle by the difference between the inspiratory and expiratory pressures; this latter quantity has been called by Diesterweg (19), the "respiratory blood." The amount of blood entering the lungs for every mm. Hg fall in mean respiratory pressure has been calculated in 11 experiments; the results may be given briefly. In the lungs from animals weighing 6.0, 9.0, 6.5, 7.5, 9.0, 8.5, 11.0, 8.5, 14.0 and 14.0 kilos (average 9.4), the blood volume increase came to 0.5, 2.0, 2.2, 2.5, 0.6, 0.9, 5.0, 2.5, 4.5, 1.5 and 6.5 c.c. (average



2.6 c.c.) respectively. Here again as in the heart-lung preparation experiments the results are extremely variable; moreover, there appears to be no correlation between the rise in blood volume of the lungs and the body weight of the animal from which they are taken.

TABLE III. Blood volume changes in the perfused lungs.

Exp.	Dog weight kilos	Change in blood flow c.c. per minute	Change in mean R.P. mm. Hg	Change in lung blood vol. c.c.
1	5.0	400 to 900		+16 + 9
2	9.0	300 to 600	-2 to -4.5	+27 +10
3	9.0	310 to 620	-1.5 to -3.5	+22 +24
			-1.5 to -5.5	

The influence of an augmentation of blood flow through the lungs on their blood volume has been determined in four experiments, Table III and Fig. 5, from which it may be calculated that, starting with a moderate

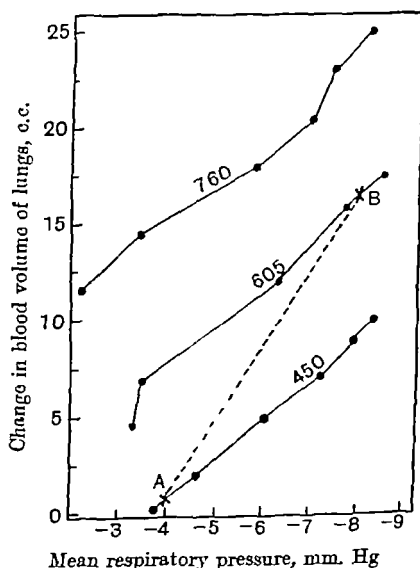


Fig. 5. Perfused lungs. Dog 11.0 kilos. The figures parallel to the curves denote the blood flow in c.c. per minute. See text.

flow, a 100 p.c. increase raises the blood content by 22, 13, 27 and 22 c.c. If the blood volume of the lungs is taken with different rates of flow over a range of respiratory pressure values, the volume increase due to

the "flow" and "pulmonary" effects can be separated, Fig. 5. In this experiment the lung volume is taken at three different flows, 450, 650 and 760 c.c. per minute over a respiratory pressure range from  $-4$  to  $-8$  mm. Hg. It will be seen that an augmentation in flow of from 450 to 760 c.c. per minute and a fall in pressure of from  $-4$  to  $-8$  mm. Hg increases the blood volume of the lungs by 24 c.c., 15 c.c. of which is due to the flow and 9 c.c. to the respiratory pressure fall. The points *A* and *B* are joined together to indicate the probable course of the volume changes in response to the respiratory pressure fall on the assumption that the output of the pump increases in the same way as the cardiac output in the heart-lung preparation. In this case the flow has been taken as increasing from 450 to 605 c.c. per minute.

#### DISCUSSION AND CONCLUSIONS.

The results obtained from the experiments on the isolated lungs show that over the range of blood flows and respiratory pressures used, alterations in flow are at least as important as changes in respiratory pressure in determining the blood volume of the lungs. A 100 p.c. increase in flow may produce the same blood volume increase in the lungs as  $4$  mm. Hg fall in respiratory pressure. In the heart-lung experiments the lung blood volume must be governed by the same principles, that is, by the blood flow which in this case is determined by the response of the heart to the negative pressure, and by the direct effect of the negative pressure on the lungs. In these experiments the peripheral flow, which is the pulmonary less the coronary flow, has been measured, and the volume of the lungs and the heart taken. It is not possible to assess the corresponding changes in the lungs, for the pulmonary flow will be larger by the coronary flow, and the lung volume changes smaller by those in the heart, than shown in Table I, columns 3 and 4 respectively. A correction would have to be made in order to compare accurately the heart-lung experiments with the isolated lung experiments; without doing this it will be seen that the series of experiments on the heart-lung preparations do not differ very widely from those on the isolated lungs if the blood volume changes for the same flow and respiratory pressure alterations are compared, from which it would appear that the heart volume response does not play an important part in the total volume changes of the heart-lung preparation provided the pericardium is intact. Such a conclusion can only be based on the average results of all experiments. The large variations in the response of the preparations have been a feature of the investigation, they no doubt

largely depend upon the initial state of distension of the pulmonary vascular bed and upon the number of the smaller vessels which are patent at the beginning of the experiment. The operative conditions previous to the setting up of the preparations have been as far as possible the same in all experiments and no reason can be assigned for the variations recorded.

It is the maximum blood volume increase in the lungs which is of the greatest interest, and there is no doubt that it would be possible for an increase in pulmonary flow of from 300 to 600 c.c. per minute to augment the blood volume of the lungs by 27 c.c. and a fall in respiratory pressure from 0 to - 5 mm. Hg to raise the volume by 30 c.c., that is, a total increase of nearly 60 c.c. as a result of the combined "cardiac" and "pulmonary" effects. These changes are well within physiological limits, although the cardiac outputs are on the low side for the dog if one accepts the figures collected from various sources by Keller(20). What fraction of the total capacity change takes place in the capillaries is as yet undecided and further work is necessary to determine this point, but it is clear that much larger volume changes in the lungs than are recorded are possible if wider ranges in heart output are taken.

#### SUMMARY.

1. In the heart-lung preparation with intact pericardium, the blood volume is increased by a reduction in respiratory (intrathoracic) pressure. The lungs are responsible for the greater portion of the volume changes, which are determined in part by the output of the heart and in part by the direct effect of the negative pressure on the lungs.

2. In the isolated perfused lungs, an increase in 100 p.c. in the blood flow produces approximately the same augmentation in the blood volume as a 5 or 10 mm. Hg reduction in the respiratory pressure.

3. The amount of blood entering the lungs for any given rise in blood flow or fall in respiratory pressure shows wide variations from one experiment to another.

The expenses of this research were in part defrayed by the Government Grants Committee of the Royal Society.

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## SOME PROPERTIES OF THE SEPARATED ACTIVE PRINCIPLES OF THE PITUITARY (POSTERIOR LOBE).

By J. H. GADDUM (*National Institute for Medical Research*).

EXTRACTS of the posterior lobe of the pituitary gland have been found to produce a great diversity of physiological effects and, in the course of time, a large number of experiments have been performed with the object of determining whether all these effects could be attributed to a single, multivalent, active principle, or whether a number of active substances were present. The former view was upheld by Abel<sup>(1)</sup> and his collaborators, while the latter has received much support from work done in this country. In a recent paper, Kamm, Aldrich, Grote, Rowe and Bugbee<sup>(2)</sup> have brought forward conclusive evidence of the separation of the substance which produces a rise in the blood-pressure of a dog from that which produces a contraction of the uterus of a guinea-pig. These workers give an account of previous work in this direction, together with the details of the method by which two separate purified preparations, "Vasopressin" and "Oxytocin," are being manufactured.

I am indebted to Messrs Parke, Davis and Co. for the opportunity of investigating further the physiological properties of these substances.

*Pressor activity.* It is stated that vasopressin contains 25 international standard units per c.c., and oxytocin less than 1 unit per c.c. These results, obtained on a dog under chloralose, have been confirmed by comparing the preparations with the British standard on the blood pressure of a spinal cat. The pressor effect of the solution prepared from 0.1 unit (*i.e.* 0.05 mg.) of the standard was indistinguishable from that of 1/250 c.c. of the vasopressin solution. The rise in blood-pressure produced by large doses of oxytocin was more prolonged than that due to small doses of vasopressin, and it seems possible that it is an effect of the oxytocic principle itself, and is not due to imperfect separation of two principles.

*Oxytocic activity.* It is stated that 1 c.c. of oxytocin contains 12 units of oxytocic activity. My estimate was 13 units per c.c. These results do not differ by a significant amount. Vasopressin contains less than 1 unit per c.c.

*Diuretic action.* The observation of Kamm and his co-workers that this effect is produced by much smaller doses of vasopressin than of oxytocin has been confirmed. The diuretic effect of large doses of oxytocin, on a cat under ether, was, like the pressor effect, more prolonged than that of small doses of vasopressin. There is as yet no evidence as to whether or not the substance which produces, under certain conditions, an antidiuretic effect can be separated from the other principles, and there is little justification for speaking, as certain authors do, of a "diuretic-antidiuretic effect." The two effects are quite distinct, and should be studied separately.

*Depressor activity.* Vasopressin showed no depressor effect on a cat, even when given in repeated doses. 1 c.c. was mixed with an equal volume of twice normal NaOH. After some hours the solution was neutralised and injected intravenously into a cat. There was no sign of pressor or depressor effect, though 0.0002 mg. of histamine produced a definite depression.

1 c.c. oxytocin (containing 12 units) showed no depressor effect in the same experiment, when similarly treated with soda, whereas 0.0005 mg. of histamine still produced a definite fall of pressure. On the other hand, when 12 units of the untreated oxytocin solution were injected into a cat, in which the blood vessels were still in high tone as the result of several large doses of vasopressin, a definite fall of blood-pressure occurred (cf. Fig. 1). The effect was equal to that produced in this cat

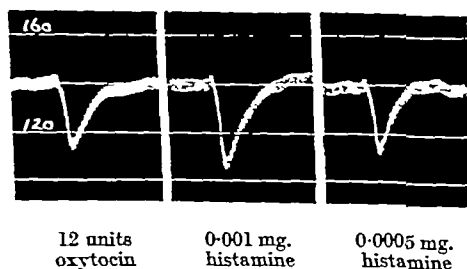


Fig. 1. Blood-pressure of spinal cat which had previously received several injections of vasopressin. Depressor effect of the intravenous injection of a large dose of oxytocin compared with that of histamine.

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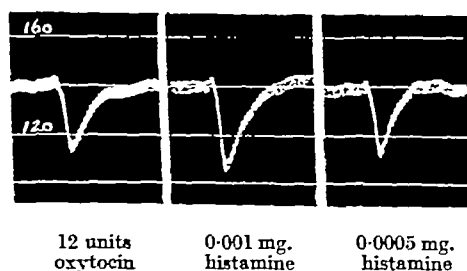


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by 0.0005 mg. of histamine, but it was evidently not due to histamine itself, since, if there had been so much histamine present, a definite fall of blood-pressure would have been produced after treatment with alkali. Similar falls of blood-pressure have been found to occur after a second

injection of Abel's purified pituitary tartrate(1). For some unknown reason the reaction is not given by all cats.

The effect is possibly analogous to the depressor action of pituitary preparations on the fowl. This was first described by Paton and Watson(3). It has been reinvestigated by Hogben(4), who has shown that the substance producing it is present in the *pars neuralis* and to a less extent in the *pars intermedia*, but not in other tissues. He also showed that it is destroyed by treatment with NaOH, and is accordingly not histamine.

It was found that a fall of arterial blood-pressure was produced in a fowl under ether by large doses both of vasopressin and of oxytocin (cf. Fig. 2). After several injections the fowl became much less sensitive,

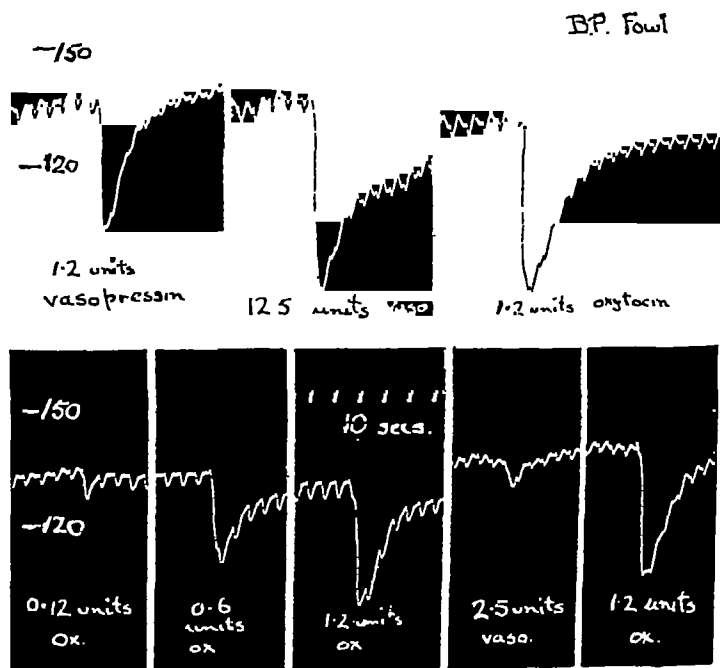


Fig. 2. Oxytocin and vasopressin both produce a fall of blood-pressure when injected intravenously in the fowl, and the preparation soon becomes much less sensitive. Oxytocin then produces the effect in much smaller doses than vasopressin.

and a direct comparison of the effects of the two preparations showed that oxytocin was active in much smaller doses than vasopressin. In

confirmation of Hogben's results it was found that this effect of oxytocin was certainly not due to histamine, which must be given in relatively large doses to produce a depressor action in the fowl. The effect also disappeared after treatment with alkali.

These two depressor actions, on the cat and on the fowl, are the only two actions, apart from that on the uterus, which have been found to be produced by smaller doses of oxytocin than of vasopressin, and it is possible that they are both due to the oxytocic principle itself. This would accord well with the fact that such effects are produced by Abel's purified preparations, in which the oxytocic titre appears to be extraordinarily high.

The depressor action on the fowl of large doses of vasopressin may also be due to the oxytocic principle, which is known to be present as an impurity.

*Action on the bowel.* Since pituitary preparations are used in the treatment of paralysis of the bowel, it was of importance to know which of these preparations was likely to be the more efficacious in this respect.

It was found that, though both preparations produced shortening of an isolated loop of the rabbit's intestine, and an increase in the peristalsis recorded by a modification of Trendelenburg's method(5), vasopressin produced these effects in smaller doses than oxytocin. The effect is rather irregular and is apt to disappear after large doses, and sometimes spontaneously. The colon appeared to be more sensitive than the ileum, and the ileum than the jejunum. In the colon there was usually

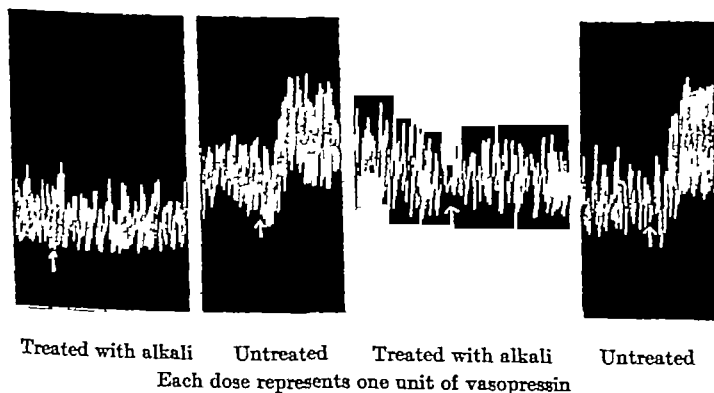


Fig. 3. Length of a strip of rabbit's ileum in 100 c.c. Tyrode's solution. Vasopressin produces an increase in the contractions and loses this property after treatment with cold NaOH.

an inhibition before the stimulation. The effect on peristalsis was more regular than that on length of a segment, and was produced by smaller doses. The substance producing these effects was certainly not histamine, because quantities of histamine, hundreds of times larger than could possibly have been present, had no such effect: further, the active substance was destroyed by treatment with cold alkali. It was not the oxytocic principle, because, although the effects were produced by comparatively small doses of the international standard, a quantity of oxytocin containing twice as much of the oxytocic principle had no effect. On the other hand, this action may be due to the pressor principle. It was found that equipressor doses of the standard and of vasopressin produced similar effects on the bowel. It was not found possible to make a precise estimate of their relative potencies.

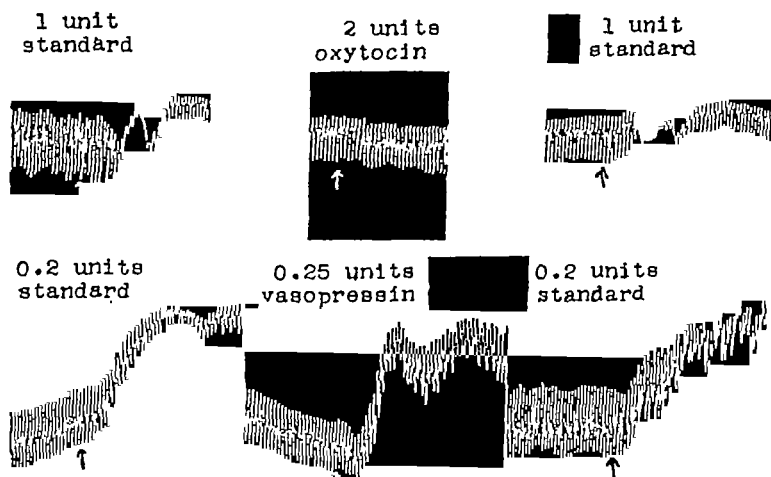


Fig. 4. Length of strips of rabbit's jejunum in 100 c.c. of Tyrode's solution. Stimulant action not due to oxytocic principle, but may be due to pressor principle.

The intravenous injection of 2 units of oxytocin in a rabbit, having a celluloid window in the abdominal wall, previously inserted by Dr Dale by aseptic operation under anæsthesia, had no appreciable effect on the intestinal movements, but an injection of 2 units of vasopressin produced a marked increase of peristalsis.

Previous work on this question is summarised in a paper by MacDonald (6), who found that pituitary preparations had little or no specific effect on the isolated cat's intestine. This observation has been confirmed.

*Dilator action on frog's melanophores.* This effect was described by Hogben and Winton(7). Vasopressin had a distinct action when 1 unit was injected subcutaneously into a frog (*R. temporaria*). One unit of the standard had a similar effect, but oxytocin had little or no action in this dose. Both substances were compared quantitatively with the

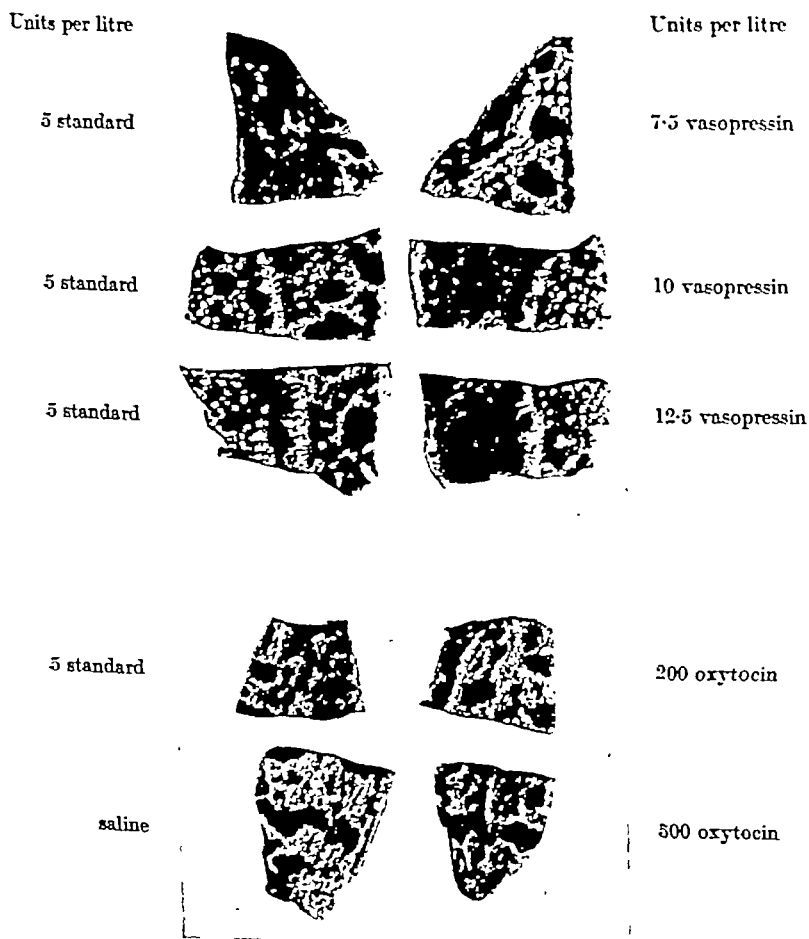


Fig. 5. Portions of skin of *Rana esculenta* after 3 hours in dilutions of pituitary. Five units of the standard have more effect than 7.5 units of vasopressin, and slightly less than 10. Two hundred units of oxytocin have less effect than 5 units of standard, but oxytocin has some effect, compared with saline.

standard by immersing pieces of frog's skin for several hours in different dilutions of the pituitary preparations in saline. The comparison of the symmetrical pieces shown in Fig. 5 showed that, in concentrations measured by pressor activity, the standard had more than 1.5 times, and slightly less than twice as much action on the melanophores as vasopressin. It also showed that oxytocin had some effect on the melanophores, but that, in concentrations measured by oxytocic action, it had less than 1/40 of the action of the standard. Identical results have been obtained in other tests, and it is thus apparent that the activity of the different preparations on the melanophores does not bear a constant proportion either to their pressor or to their oxytocic activity. This confirms the results of other workers, which have shown that the action on the melanophores is due to a separate substance. A. J. Clark<sup>(6)</sup> found that this substance does not pass so readily as the others through collodion membranes. Several workers<sup>(7, 9)</sup>, have found that it occurs in relatively higher concentration in the pars intermedia than in the pars neuralis. It appears to have been almost completely excluded from oxytocin, but in vasopressin it has been concentrated to about one-half the same extent as the pressor principle itself.

#### SUMMARY.

"Oxytocin" and "Vasopressin" have been subjected to a more detailed physiological study than that accorded them by their makers, and various conclusions of physiological importance have been drawn.

"Oxytocin" has, in addition to its action on the uterus, a depressor action on the fowl and, in certain circumstances, on the cat.

"Vasopressin" has, in addition to its effects on the blood-pressure and on the kidney, a specific stimulant action on the bowel of a rabbit and a dilator action on the melanophores of the frog. The latter effect is apparently due to a different principle, so that vasopressin is not yet a physiologically pure preparation.

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# VASOMOTOR EFFECTS OF STIMULATING THE RIGHT SPLANCHNIC NERVE.

By J. H. THOMPSON.

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MOST of the investigations relating to the form of the splanchnic blood-pressure curve have been carried out upon the nerve on the left side. Apparently, little attention has been devoted to a systematic study of the responses from the right nerve. The present paper describes how, as the result of such a study, effects are obtained which cannot be obtained with the left nerve.

As is well known, on stimulating the left nerve the vascular response gives in certain conditions indications of a depressor as well as the usual pressor action. In the course of this work many experiments have been done on the left nerve: but these, in that they confirm previous work (1-7), served in the main only to bring out the contrast with the experiments on the right nerve. With regard to them, therefore, it is sufficient to make here the following observations:

(1) In ether anaesthesia, under urethane or luminal sodium, the dip in the blood-pressure tracing that occurs between the primary and secondary rise of pressure diminishes on repeated stimulation of the left



Fig. 1. Diminution of the "dip" under ether anaesthesia by repeated stimulation of the left nerve.

nerve (Fig. 1) and finally disappears as it does when the adrenal veins are tied, as Vincent and Wright showed (8). Under chloralose, even



when the adrenal veins are tied, in confirmation of observations of Vincent and Wright(5), this feature tends to persist (Figs. 2 *a* and 2 *b*);

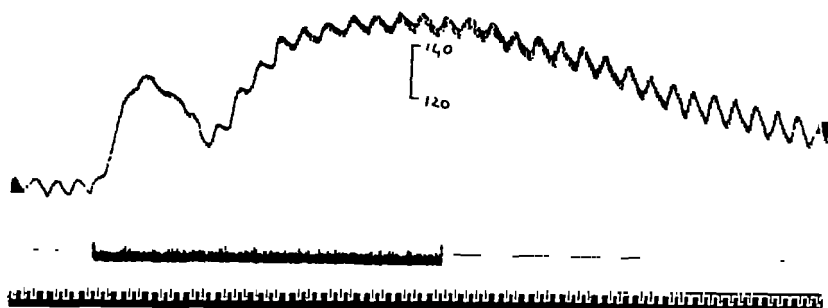


Fig. 2 *a*. Chloralose—adrenal veins open. Stimulation of left nerve.

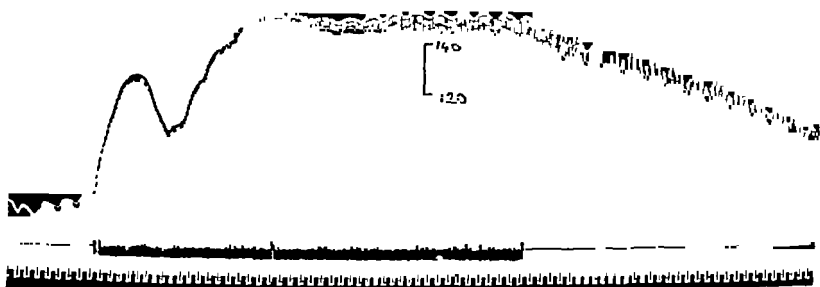


Fig. 2 *b*. Chloralose—adrenal veins tied. Stimulation of left nerve.  
Note persistence of “dip.”

this anæsthetic apparently increasing the sensitivity to adrenaline so that amounts, otherwise ineffective, which leak through some collateral circulation have a depressor action.

(2) In decerebrate preparations after the effect of the anæsthetic has passed off the dip in the curve does not occur(7), but a definite augmentation of heart rate and force results in a secondary rise superimposed on the primary; after tying the adrenal veins this “step” on the tracing is not seen.

Peculiarities that have been observed in the effects of stimulating the left nerve under chloralose will form the subject of another paper (Vincent and Thompson(9)).

## METHODS.

The nerves were exposed in the abdominal cavity, care being taken to avoid unnecessary exposure and handling of the intestines. The nerves were divided, and the peripheral ends stimulated. The stimulus consisted of an induced current rapidly interrupted by a Neef hammer.

It is important to note that the stimulations were made at short intervals, *e.g.* as soon as the blood-pressure returned to the mean, the next stimulus was given. If an interval elapses between stimulation, it may prove to be sufficient for recovery of the vaso-constrictor fibres. This is probably one of the reasons why the few workers who have used this nerve for experimental purposes have failed to show the results here recorded.

Also, in order to produce the exhaustive effect, the stimulus must be adequate.

The first stimulation of the right splanchnic nerve usually effected a rise of blood-pressure identical with that obtained from the left nerve, except that there was a distinct tendency for the blood-pressure to rise at the end of stimulation, no matter at what position in the curve it ceased.

But, with later stimulations, a marked difference became apparent, and was shown in three ways.

1. After about four successive and equal stimulations, at short intervals, it was impossible to obtain any rise of blood-pressure upon application of the same strength of stimulus (Fig. 3). Apparently, there



Fig. 3. Exhaustion of pressor response in right nerve by successive stimulation of equal strength at short intervals. Note increase of after-rise as exhaustion occurs.

was a rapid exhaustion of the vaso-constrictor fibres in the nerve. The exhaustion (as evidenced by the decrease in height of the curve) although rapid, was uniform. Occasionally, however, a tendency to increase in height, followed by a very quick exhaustion, has been observed. A large number of experiments have failed to show similar effects on the left

nerve. Even after thirty stimulations, no such exhaustion can be produced.

2. Coincident with the diminution of the rise after the first stimulation, a remarkable kind of rise was found to occur at the moment when stimulation was concluded. It consisted of a very rapid rise with a gradual decline (Fig. 4). It took place irrespective of the point on the curve at which stimulation ceased (Fig. 5) and was equal in size in all positions. It became more marked as the general rise decreased (Fig. 3).

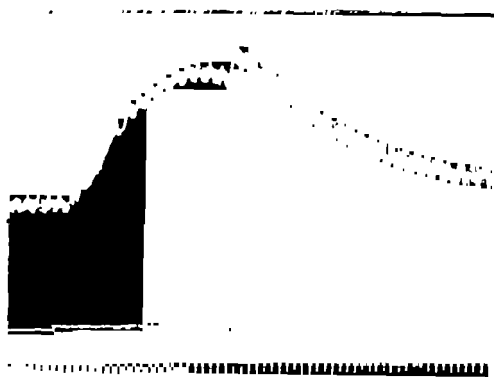


Fig. 4



Fig. 5

Fig. 4. Luminal sodium. Rapid rise on cessation of stimulation at height of splanchnic rise. (Right nerve.)

Fig. 5. Ether—rapid after-rise at end of splanchnic curve due to stimulation of right nerve.

Tying the adrenal veins did not affect it, nor was it abolished by clamping the superior mesenteric or portal veins, and it was equally obtainable when the clamps were removed. Removal of the clamp was attended by a small immediate fall of blood-pressure, succeeded by a prolonged rise and decline to the mean pressure.

3. Further stimulation of the right nerve with the same strength and frequency of current after exhaustion of the rises had occurred, resulted in a pure fall of blood-pressure. The fall commenced at the moment of stimulation, rapidly reached its maximum depth, and maintained this depth for a very considerable period as long as stimulation was made (Fig. 6). On cessation of stimulation, a rapid rise was obtained comparable to that described above, and the rise was continued beyond the mean blood-pressure, eventually describing a long gradual return to the original level.

Application of a stronger stimulus at this stage produced a rise of blood-pressure, whilst a weaker stimulus caused a marked fall.



Fig. 6. Decerebrate cat—fall of blood-pressure with right nerve, showing persistence of the effect as long as stimulation continues.

Ultimately, the strongest stimulus elicited falls, and it became impossible to obtain pressor responses at all (Fig. 7). A curious phenomenon

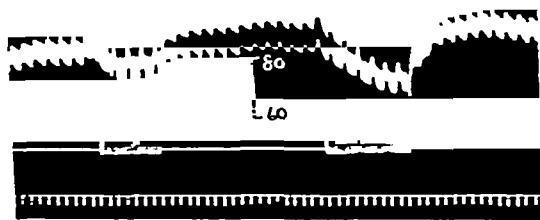


Fig. 7. Luminal sodium. Effect of weak and strong stimuli on right nerve after repeated stimulation. Falls of blood-pressure with all stimuli. First stimulus—very strong. Second stimulus—weak.

constantly observed was that the depth of the fall was inversely proportional to the strength of the stimulus. The most effective means of producing falls have been mechanical: merely touching the nerve with the electrodes, or gentle stroking with a smooth glass rod caused marked falls of blood-pressure (Fig. 8). Stretching the nerve had little effect.

Sometimes if an interval of rest was given to the nerve the effect of exhaustion leading to a fall upon stimulation disappeared temporarily,

and was substituted by a rise; but if the nerve was stimulated frequently and many times, no such recovery was experienced although two hours were allowed to elapse.

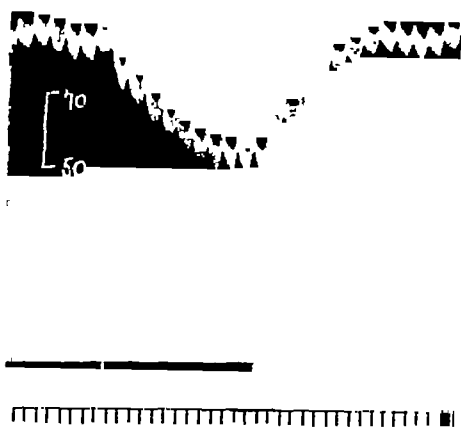


Fig. 8

Fig. 8. Luminal sodium. Effect of stroking right nerve with glass rod.

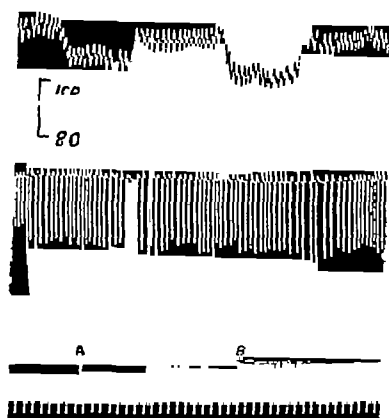


Fig. 9

Fig. 9. Decerebrate cat—stimulation of right nerve. *A*—adrenal veins open. *B*—adrenal veins tied.

These results have been repeated with the adrenal veins tied, and no difference noted except perhaps that the fall was more easily induced (Fig. 9).

Repeated attempts to elicit these phenomena with the left nerve have completely failed.

Identical results have been obtained with the intact animal under various anæsthetics, and in the decerebrate preparation with or without anæsthetics. Under ether rather more stimulations (7 or 8) are required to effect exhaustion of the usual splanchnic rise. In pithed animals, in spite of the low blood-pressure present, the phenomenon was easily produced.

These results lead to the conclusion that the right splanchnic nerve contains not only vaso-constrictor fibres, but also a considerable number of vaso-dilator fibres.

In 1889 Bradford(s) obtained evidence of vaso-dilator fibres in the splanchnic nerves. Apparently either nerve was used indiscriminately.

He found that slight falls of blood-pressure could be produced, but only when the rate of excitation was slow—1 per second: quicker rates caused rises of blood-pressure. I have failed to confirm these results for the left nerve, although very slow rates of excitation have been employed, and the falls obtained with the right nerve have originated from a rapidly interrupted current.

The rapid after rise is also evidence of a mixed nerve, the vaso-constrictor effect being prolonged beyond the vaso-dilator. It cannot be due to any disturbance in the hepatic circulation since clamping the portal vein does not alter it.

It is interesting to note that the right splanchnic nerve is usually of much greater diameter than the left, and contains many more fibres. If traced centrally, it is found to be formed by the union of two (occasionally three) nerves, which join a short distance before entering the ganglion as one trunk. Sometimes there is a failure to unite and the two nerves (each about the average size of the left nerve) run parallel and join the ganglion separately. In such circumstances, stimulation of the main nerve gives results corresponding to those obtained above, excepting that the exhaustive process is longer and the falls of pressure smaller, whilst stimulation of the accessory splanchnic nerve produces very small rises of blood-pressure and depressor effects rapidly supervene.

This seems to indicate that the accessory splanchnic contributes most of the dilator fibres found in the common trunk. On the left side, a small nerve is present, which is a branch of the main nerve, and which always enters the ganglion separately. There is no anatomical distribution comparable to that obtaining on the right side.

#### SUMMARY.

Experiments performed on the right splanchnic nerve have revealed the presence of a considerable number of vaso-dilator fibres in it. Stimulation of the nerve has shown that its pressor response can be rapidly exhausted, that a sharp after-rise occurs on cessation of stimulation, and that depressor responses can be easily elicited.

Allusion is made to certain anatomical differences between the right and left nerves.

It is a great pleasure to express my thanks to Prof. Swale Vincent for his advice and assistance in the conduct of this research.

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# THE PHARMACODYNAMICAL ACTION OF CHLORALOSE.

By SWALE VINCENT AND J. H. THOMPSON.

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Medical School.)

*Introduction.* Chloralose was introduced some years ago by Richet for experiments on animals. The drug does not appear to have been very extensively used as an anæsthetic for such experiments, although, as we shall show, it possesses several obvious advantages. The chloralose used in our experiments was obtained from Messrs Baird and Tatlock, Ltd., its origin being French. We have been informed that it is prepared by heating an anhydrous mixture of chloral and glucose at 100° C. for about one hour. The residue is treated with a little water and then boiling ether; the toxic isomer, parachloralose is eliminated by crystallisation. The formula is  $C_8H_{11}O_6Cl_3$ .

A solution saturated at 40° C. was made and injected intravenously.

All the experiments have been performed upon decerebrate cats, after all the ether has been expired.

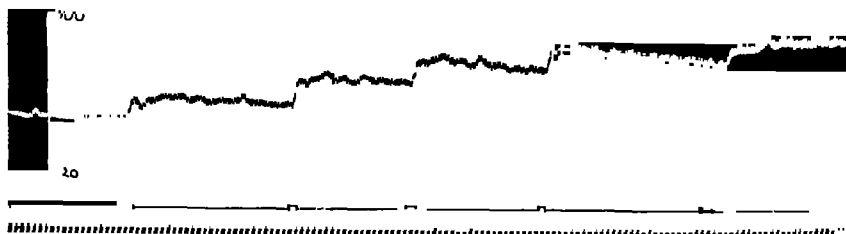
*Action on the mean arterial blood-pressure.* 10 c.c. of the above solution injected intravenously is sufficient to raise the blood-pressure approximately 20 mm. of Hg. The effect is not observed immediately, but follows upon the initial rise caused by the saline. The blood-pressure remains raised to its new level for a considerable time, and by means of repeated injections can be stepped up to more than twice the original height (Fig. 1).

Control experiments have been carried out with glucose solution, and with chloral preparations, and since under certain conditions normal saline solution will produce considerable rises of pressure, we have performed numerous controls with this solution also. All of these have failed to produce the results obtained with chloralose. It is significant that intact animals anæsthetised with chloralose always have a high initial blood-pressure.

*Action on the somatic nerves.* Stimulation of the sciatic and anterior crural nerves with the strongest available stimuli failed to elicit any



reflex responses when the animal was under chloralose. Weak stimuli were equally ineffective. This inhibitory action of chloralose is manifested quickly and can be shown five minutes after the first injection.



*Time tracings indicate periods of 5 seconds.*

Fig. 1. Cat: decerebrate. Intravenous injections of 10 c.c. of chloralose solution (sat.  $40^{\circ}$  C.) resulting in raising the mean blood-pressure from 40 mm. Hg to 90 mm. Hg. Note the "stepping up" effect.

*Action on depressor reflexes.* Vaso-motor reflexes of a depressor character obtained by scratching the skin, or kneading the muscles or intestine are considerably augmented after administration of chloralose. This is particularly marked in the case of the intestine; indeed, the increased sensitivity of the vaso-motor response is so great that falls of blood-pressure occur when the gut is kneaded intra-abdominally before



Fig. 2. Cat: decerebrate. (a) Intra-abdominal kneading of intestine showing initial mechanical rise followed by a marked secondary rise due to autacid pressor substance. (b) 10 c.c. chloralose (sat.  $40^{\circ}$  C.) administered intravenously 5 minutes before stimulation. Initial vaso-motor fall although kneading of intestine is intra-abdominal. Secondary rise still present. (c) 15 min. later, vaso-motor fall alone remaining.

the normal pressor effect due to the autacid substance (Vincent and Thompson<sup>(1)</sup>) (Fig. 2). The hypersensitivity of nerve terminals, especially

those concerned in reflex dilatation of the pupil, has been observed by McDowall(2) under chloralose anæsthesia in intact animals.

*Action on the splanchnic nerve.* Stimulation of the peripheral end of the divided left splanchnic nerve in the abdomen after an injection of chloralose results in a remarkable exaggeration of the whole curve of blood-pressure. It becomes noticeable 5 minutes after injection, and reaches its maximum 25 minutes later. Repeated injections still further increase the rise, so that eventually rises nine or ten times the height of the original rise are obtained with the same strength of stimulus (Figs. 3*a* and 3*b*). We have observed that the increase in height of the

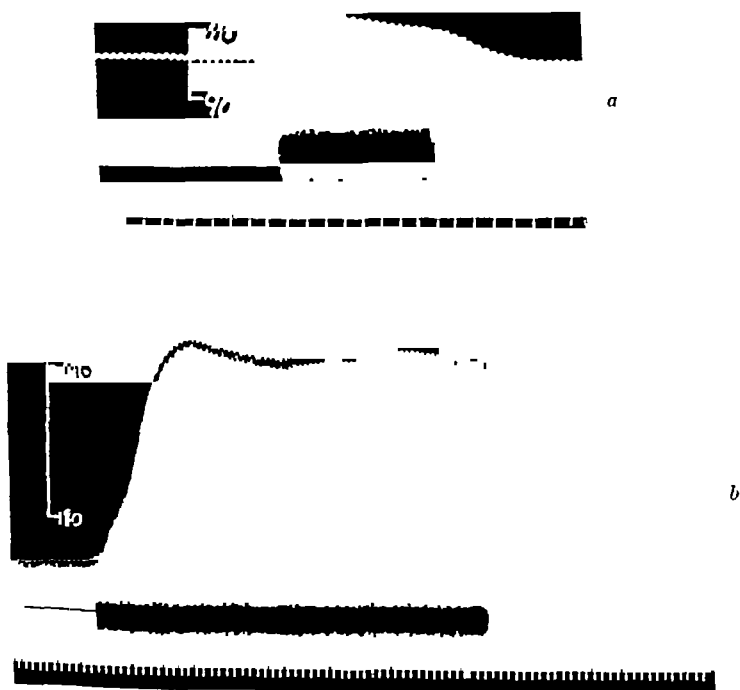


Fig. 3*a*. Cat·decerebrate Stimulation of left splanchnic nerve before injection of chloralose.

Fig 3*b* Stimulation of left splanchnic nerve with same strength of current 1 hour after two intravenous injections of 10 c c chloralose solution (sat 40° C ).

curve is greater at the end of half an hour if the nerve is stimulated at intervals of about 5 minutes than if it is left undisturbed during this period.

Division of both vagi in the neck made no difference to the results, and thus a parasympathetic action may be excluded.

*Interaction of the adrenal glands.* If the adrenaline veins are clamped, and while the clips are in position the left splanchnic nerve is stimulated, whether chloralose was given before or after putting them on, the effect on blood-pressure is no greater than it is without chloralose. Clearly the presence of the drug in the vessels on which the splanchnic acts does not by itself bring about any increase of the effect. But if the chloralose is injected and allowed to circulate for a short time before the veins are clamped, then when later the clips are taken off a remarkable effect is observed. As the blood from the gland is released into the circulation, a rise of blood-pressure occurs spontaneously which may be very large if the amount of chloralose given was large (Fig. 5). If, on the other hand, the veins were clamped before giving the chloralose, so that owing to the arrested circulation in the glands the drug does not effectively reach them, then when the clips are taken off there is but little rise of pressure. This suggested that chloralose acts in the adrenal gland so as to increase the amount of adrenaline produced in it. And accordingly the following experiment was done: a small amount of chloralose, 0.25 c.c. of the solution, was injected directly into the gland, and then after extirpation of both the semi-lunar ganglia, the post-ganglionic fibres to the gland were stimulated, and the effect shown in Fig. 6 was obtained, a considerable rise of blood-pressure.

It is true the sudden descent of the tracing when the stimulation stopped is difficult to account for, but the result, taken together

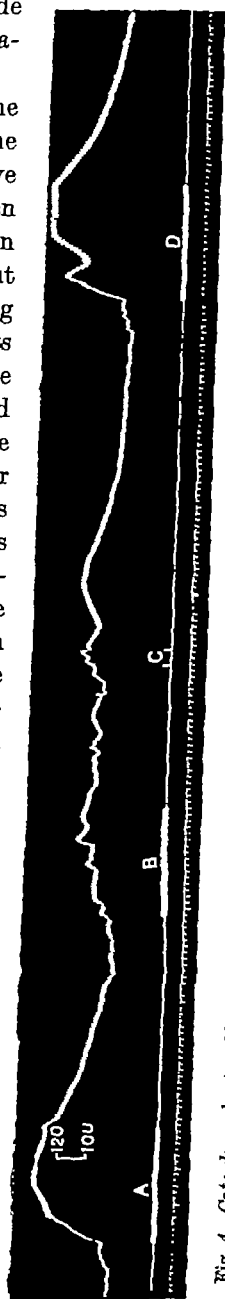


Fig. 4. Cat; decerebrate. 10 c.c. chloralose (sat. 40° C.) 30 min. previously. (A) Stimulation of left splanchnic nerve with adrenal veins open; (B) with veins clamped; (C) removal of clamps followed by rise, (D) stimulation with veins open. Same strength of stimulation used for (A), (B) and (D).

with the effect of releasing the blood from the glands when chloralose was given before obstructing the circulation, is in favour of an increased formation of adrenaline under the influence of the drug.

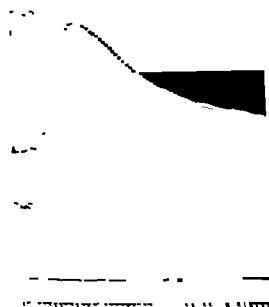


Fig. 5

Fig. 5. Cat: decerebrate. 10 c.c. chloralose (sat. 40° C.). 20 min. previously. Large rise of blood-pressure consequent upon release of clips on adrenal veins after four stimulations of left splanchnic nerve with veins clamped.

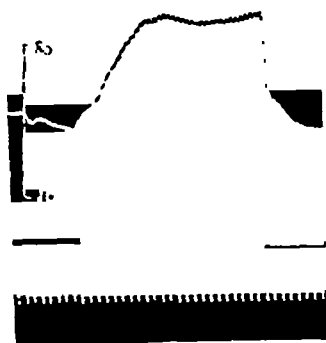


Fig. 6

Fig. 6. Cat: decerebrate. Semi-lunar ganglia removed. 0.25 c.c. chloralose (sat. 40° C.) injected directly into the left adrenal gland. Stimulation of fibres from semi-lunar ganglia to the gland. (Prior to injection only very slight rises could be obtained.)

*Injection of adrenaline.* When a mixture of chloralose solution (saturated at 40° C.) and adrenaline solutions of various strengths is intravenously injected, a large and long-sustained rise of blood-pressure is caused instead of the ordinary transitory rise obtained by injection of adrenaline alone. The persistence of the rise is remarkable, and when doses of 0.1 mg. of adrenaline are given, the maximum height may be maintained for longer than ten minutes (Fig. 7).

A similar result is effected when adrenaline is administered to an intact or decerebrate animal under chloralose.

If adrenaline be injected intravenously whilst this sustained rise is in progress, falls of blood-pressure occur (Fig. 8). It appears to be impossible to produce a pressor response by means of adrenaline when this rise is present. Even large doses of adrenaline—2 or 3 mg.—result in falls.

#### DISCUSSION OF RESULTS.

The experiments relating to clamping the adrenal veins clearly demonstrate that chloralose activates the secretion of adrenaline. Precisely how this is done is not obvious, but it appears to be an action

on a local mechanism consisting of the gland itself and possibly the nerve-fibres reaching it from the semi-lunar ganglia, as evidenced by the large

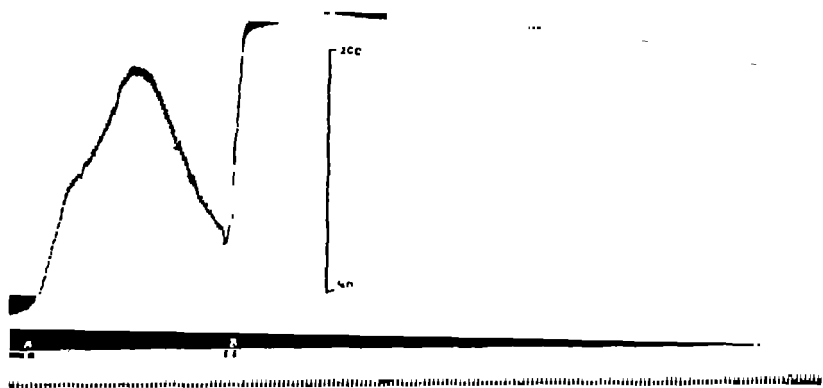


Fig. 7. Cat: decerebrate. (a) Injection of 0.1 mg. adrenaline. (b) Injection of 0.1 mg. adrenaline and 10 c.c. of chloralose (sat. 40° C.). (The pressure was maintained at a high level for 10 minutes longer than shown in the above figure.)

rise of blood-pressure elicited from stimulation of the post-ganglionic fibres to the gland after chloralose has been injected into it and the ganglia removed.



Fig. 8. Cat: decerebrate. Sustained rise of blood-pressure produced by injection of adrenaline plus chloralose. Injection of adrenaline of various strengths: (a) 0.01 mg.; (b) 0.2 mg.; (c) 0.01 mg.; (d) 0.005 mg.—during the rise caused falls of blood-pressure.

This explains the increase in the size of the splanchnic curve, the diminution after clamping the adrenal veins confirming such an explanation. Now it is found that the rise of blood-pressure produced by stimulation of the left splanchnic nerve 30 minutes after injection of chloralose

is greater if stimulation be applied to the nerve during this interval. This seems to indicate that the ultimate products of each stimulation sensitise the secretory mechanism in the gland for the next stimulation. It may be a direct effect upon the nerve terminations, or a catalytic action upon the precursor of adrenaline produced by stimulation of the nerve fibres.

In support of this theory is the fact that adrenaline is stabilised by chloralose, as shown by the long sustained rises. Hence the permanent raising of the mean blood-pressure. Possibly a weak chemical compound is formed between chloralose and adrenaline, which is more stable than adrenaline. This compound sensitises the nerve terminals. We think that this is the interpretation of the hypersensitivity of the nerve terminals in other parts of the body, and it is significant that the effect is most marked at sympathetic terminals.

#### SUMMARY.

Experiments have been performed upon decerebrate cats under the influence of chloralose to demonstrate the action of the anæsthetic upon the mean blood-pressure, the somatic, nerves, nerve terminals, and the left splanchnic nerve, and to show the effects of injection of adrenaline.

Reasons are advanced for the belief that chloralose stimulates the production and discharge of adrenaline from the adrenal gland. We have also given evidence for the stabilisation of adrenaline in the body, as, for example, that injected into the blood, by the formation of a stable compound. This compound sensitises nerve terminals and tends to raise permanently the mean blood-pressure.

The expenses of this research have been partially defrayed by grants to one of us (S V.) from the Royal Society and the British Association.

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## THE EFFECT OF ERGOTOXIN ON PHLORRHIZIN GLYCOSURIA.

BY A. B. ANDERSON AND M. D. ANDERSON.

(*From the Biochemical Laboratory, Cambridge.*)

IN a previous paper<sup>(1)</sup> a report was made on the effect of atropine, ergotamine, and pituitrin on phlorrhizin glycosuria. In this work the authors administered tyramine in the experiments purporting to show the effects of ergotamine, believing at the time that the ergotamine mentioned in the literature was the tyramine of ergot extracts. Our mistake having been pointed out to us, we have repeated the experiments using the alkaloid ergotoxin, and the results are reported below. We feel justified in using ergotoxin, since Dale and Spiro<sup>(2)</sup> have shown that the pharmacological effects of ergotoxin and ergotamine are indistinguishable.

The technique was the same as that described in our first paper (*loc. cit.*). Ergotoxin in the form of the phosphate<sup>1</sup> was dissolved in water and administered to rats by subcutaneous injection. Some of the results are given in the following table. All the animals were fully phlorrhizinised for three or four days before the first day given in the table.

The figures show in all cases a rise in the D/N ratio and percentage of glucose after the administration of ergotoxin, and at the same time there is a fall in urine volume, total sugar and total nitrogen. In some cases the rise in D/N ratio persisted for two days, after which it returned to the normal figure. Judging from these results, ergotoxin has no action antagonistic to that of phlorrhizin. The effect on the D/N ratio is similar to that reported for atropine in our first paper (*loc. cit.*), and the objections which we raised there to the findings of Teschendorf<sup>(3)</sup> with ergotamine would appear to be justified. We cannot subscribe to the theory that phlorrhizin acts on the sympathetic nerve endings of the kidney.

<sup>1</sup> Ergotoxin phosphate supplied by British Drug Houses, Ltd.

Rat and weight before exp.	Day No.	Urine vol. c.c.	Glucose p.c.	Glucose total gm.	Nitrogen per c.c. mg.	Nitrogen total mg.	D/N	Dose of ergo-toxin phosphate mg.
<i>EK</i> 215 gm.	1	52	4.98	2.59	18.8	977	2.05	
	2	57	5.58	3.17	10.2	1094	2.9	
	3	77	4.40	3.43	17.6	1355	2.5	
	4	35	5.88	2.06	19.0	665	3.1	10
	5	56	5.37	3.01	15.9	890	3.4	
	6	70	4.80	3.36	16.7	1169	2.9	
<i>EL</i> 195 gm.	1	59	4.42	2.61	17.8	1050	2.5	
	2	48	5.34	2.56	19.7	946	2.7	
	3	63	4.49	2.83	17.7	1116	2.5	
	4	43	5.93	2.55	18.5	795	3.2	5
	5	53	5.01	2.65	17.4	922	2.9	
	6	63	4.41	2.78	17.3	1090	2.55	
<i>EM</i> 170 gm.	1	48	4.74	2.27	17.6	845	2.7	
	2	47	4.56	2.14	16.6	780	2.75	
	3	49	5.05	2.47	17.5	858	2.9	
	4	31	6.59	2.04	18.3	567	3.6	5
	5	47	5.79	2.72	19.0	893	3.05	
	6	48	5.58	2.68	19.3	926	2.9	
	7	45	5.70	2.56	19.9	896	2.9	
	8	35	6.56	2.30	19.8	693	3.3	10
	9	48	6.00	2.88	17.6	845	3.4	
	10	54	5.32	2.87	17.5	945	3.0	
<i>EO</i> 222 gm.	1	55	4.35	2.39	16.9	930	2.6	
	2	41	4.95	2.03	18.4	754	2.7	
	3	52	4.14	2.15	16.2	842	2.55	
	4	57	4.38	2.50	16.2	923	2.7	
	5	37	5.10	1.89	16.9	625	3.0	10
	6	55	4.71	2.59	16.2	891	2.9	
	7	67	4.78	3.20	17.2	1152	2.8	
	8	66	4.83	3.19	16.9	1115	2.9	
	9	51	5.26	2.68	16.9	862	3.1	15
	10	60	4.72	2.83	16.9	1014	2.8	
	11	61	5.16	3.15	17.8	1086	2.9	

# SUMMARY.

1. An error in a previous paper in which tyramine was administered for ergotamine is corrected by repeating the experiments with ergotoxin.
2. Ergotoxin causes a rise in the D/N ratio and percentage of sugar in the urine of fully phlorrhizinised rats on a protein-fat diet.
3. The evidence from these experiments does not support the theory that phlorrhizin produces glycosuria by acting on the sympathetic nerve endings of the kidney.

We wish to thank Dr H. H. Dale and Prof. Lovatt Evans for information and advice, and Prof. Sir F. G. Hopkins for his kind interest in this work.



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2. Dale and Spiro. *Arch. f. exp. Path. u. Pharm.* 95. p. 337. 1922.
3. Teschendorf. *Klin. Wochensch.* 3. p. 1811. 1924.

NOTE. Since submitting this paper for publication we have obtained similar results in a series of experiments in which we used "ergotamine" tartrate kindly supplied by "Sandoy."

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# PROCEEDINGS

## OF THE

# PHYSIOLOGICAL SOCIETY,

### *January 21, 1928.*

**The energetics of plain muscle.** By L. E. BAYLISS (*Sharpey Scholar*).  
(*From the Department of Physiology and Biochemistry, University College, London.*)

It is generally assumed that the measurements of the oxygen consumption of plain muscle have proved that the tonic contractions could not possibly be maintained by a continuous stimulation analogous to a tetanus in striped muscle, since too little energy is available. No account, however, has been taken of the very much slower relaxation of plain muscle than of striped muscle, and it is quite reasonable to suppose that this might be a very important factor, since the slower a muscle relaxes, the fewer contractions are required per unit time to produce a fusion.

We can, moreover, easily calculate the order of the energy requirements of a muscle relaxing to half its maximum tension in 10 secs., for example (very few plain muscles relax more rapidly than this), by extrapolation from the measurements of Hill and Hartree(1) on the relaxation time of a frog's sartorius muscle, and from those of Hartree and Hill(2) on the rate of heat production of the same type of muscle maintaining 1 grm.-cm. potential energy. The relaxation time of a frog's sartorius at 35° would be about  $3.3 \times 10^{-2}$  secs. and its initial heat production per grm.-cm. per sec. would be about  $7.5 \times 10^{-5}$  cals., so that if the rate of stimulation is proportional to the rate of relaxation and the heat production per contraction is the same in plain muscle as in striped, we can calculate that a plain muscle should produce about  $7.5 \times 10^{-5} \times \frac{3.3 \times 10^{-2}}{10}$  which is  $2.5 \times 10^{-7}$  cals. per grm.-cm. per sec.

Hence the total heat production, which is twice the initial heat production, would be  $5 \times 10^{-7}$  cals. per grm.-cm. per sec.

Now one of the most powerful of plain muscles is the retractor penis of the dog, and we should calculate, on this basis, that a muscle of this type 5 cm. long, maintaining a tension of 20 grm., would produce about  $5 \times 10^{-5}$  cals. per sec., whereas Lovatt Evans(3), working on a

variety of plain muscles, recorded isotonically, so that their tensions were small, has observed an oxygen consumption of around 0.15 ml. per hour, which reduces to about  $20 \times 10^{-5}$  cals. per sec., four times as great as that calculated on the assumption that the tension is maintained by means of a continuous stimulation.

These considerations enable one to resolve at once the dispute as to whether the metabolism of a plain muscle is increased or decreased when the tone is increased. If the energy expenditure is determined by the potential energy of the muscle, we should predict that when the muscle is lengthened, or increases its tension, the metabolism would increase, whereas if it is allowed to shorten under constant tension, the metabolism would decrease. This is just what has been observed. Cohnheim(4), who increased tone by hanging a weight on the muscle, finding an increased oxygen consumption, and Lovatt Evans(3), who increased the tone with drugs isotonically, a decreased. It is interesting to note, moreover, that in Lovatt Evans' series of measurements the heavier muscles had a greater oxygen consumption than the lighter ones. This, also, could be predicted on theoretical grounds, since the heavier muscles would have been longer. The increased cross-section would, of course, reduce the stress per muscle fibre and increase the number of fibres under stress proportionally, so that this factor can be neglected in calculating the metabolism.

It is clear, therefore, that no evidence in favour of a special tonus mechanism in plain muscle can be obtained at present from energy considerations.

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3. Evans, C. Lovatt. *This Journ.* 58. p. 22. 1923.
4. Cohnheim, O. *Ztsch. f. Physiol. Chem.* 76. p. 298. 1912.

# PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY, *February 18, 1928.*

**Vagus inhibition in relation to ions.** By B. FINKLEMAN.  
(*Manchester.*) (*Preliminary communication.*)

Changes in the composition of the perfusing fluid may alter the inhibitory power of the vagus on the heart, but there is much conflicting evidence in the literature on the subject. The usual method of sinus perfusion is unsuitable in such investigations since conditions are constantly altering during the experiment. A series of experiments have been performed upon the Loewi isolated heart vagus preparation of the frog. In a good preparation the vagus threshold is very sharp and does not tend to shift spontaneously.

It was found impossible to obtain any change in the vagus threshold by increasing the potassium content of the perfusate. This is in contradiction to the work of Howell(3) who stated that the vagus sensitivity was increased 25-30 p.c. by increasing the concentration of potassium in his Ringer's solution. It has been found, however, that a quite definite increase of the vagus sensitivity takes place on increasing the calcium content of the Ringer. This change, unlike the almost instantaneous effect on the amplitude of contractions, often only appeared after a few minutes, which suggests that absorption was necessary prior to its appearance.

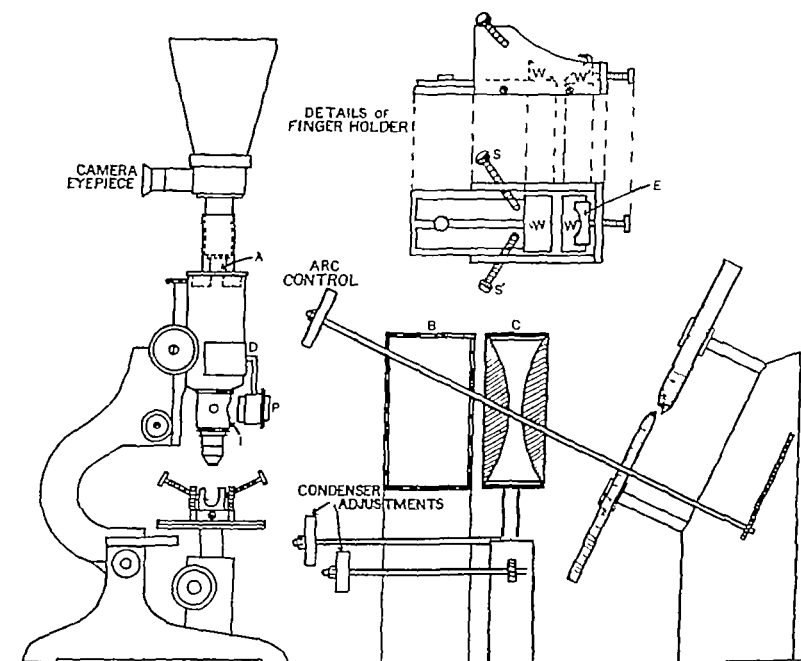
There appears to be evidence of the existence of two mechanisms concerned with vagus inhibition in the heart. When, as in the experiments of Burridge(1) and Colle(2), the sino-auricular junction is stimulated directly, increase of potassium or decrease of calcium enhances the inhibitory effect, which is antagonised by increased calcium or decreased potassium. When the vagus nerve itself is stimulated, its effects are intensified by an increase in the calcium content of the perfusate and are relatively unaffected by increase of potassium. It may be that calcium is concerned with the transmission of the vagus impulse to the nerve endings while potassium acts on the nerve endings themselves.

1. Burridge. *This Journ.* 51. p. 45. 1917.
2. Colle. *Compt. rend. Soc. Belge de Biol.* 94. pp. 786 and 1257. 1926.
3. Howell. *Amer. J. Physiol.* 15. p. 280. 1906.

**Apparatus for the observation and photography of the skin capillaries in man.** By G. L. BROWN and F. W. LAMB.

The apparatus described is a simplified form of that used by Crawford and Rosenberger<sup>1</sup> in their observations on the capillaries of man by means of the cinematograph.

The method employed is to illuminate the finger vertically, avoiding the glare of the directly reflected light by means of polarisers.



A carbon arc lamp was used as illuminant; it carries a positive cored carbon of 1.5 cm. diameter and a negative carbon, copper cored and coated, of 1 cm. diameter. A current of 7 amp. was used for observation and 16 amp. for photography. The light passes through the condenser (C), focal length 8 cm., which can be adjusted horizontally and vertically to compensate for movements of the arc, and through the large water-bath (B), 7 cm. thick.

The microscope carries on a clip (D) a substage polariser (P) with its back lens removed; it can be rotated through 180°.

<sup>1</sup> Crawford and Rosenberger. *Journ. Clin. Investigation* 2. p. 343. 1926.

The light passes through the polariser and enters a prism type vertical illuminator (*I*); the cover-glass type may be used but is not so satisfactory. The draw tube of the microscope is removed and replaced by the rotating eyepiece analyser (*A*). A Leitz "Micca" micro-camera is used for taking the photographs.

Since focussing by the coarse adjustment of the microscope throws the apparatus out of line, the stage of the microscope is removed and replaced by a rack and pinion stand which is let into the bench.

The stand is fitted with a mechanical stage carrying the finger-holder. The finger-holder is arranged so that the ball of the finger rests between the adjustable wedges (*W* and *W'*); the curved plate (*E*) rests under the tip of the nail and prevents vertical movements of the finger. The finger is steadied by the two screws (*S* and *S'*) which press gently just behind the terminal interphalangeal joint. This arrangement of the finger-holder is for observing the capillaries of the nail bed; for observation of the capillaries of the dorsum of the phalanges the U-shaped plate, as shown in the large drawing, is used. The arm of the subject is supported on sand-bags and clove oil is used on the finger. Recently we have used a weak alcoholic solution of dicyanin, a dye with an absorption band between red and yellow, as a light filter and find that it increases the visibility and ease of observation of the capillaries considerably, without increasing the length of exposure for photographs.

Using Imperial 650 H and D plates, exposures of  $\frac{1}{3}$  -  $\frac{1}{2}$  sec. give plates of good density.

As Crawford and Rosenberger point out a far greater degree of detail can be made out by this method than by means of oblique illumination.

#### Class experiments in leucocytosis. By R. J. S. McDOWALL.

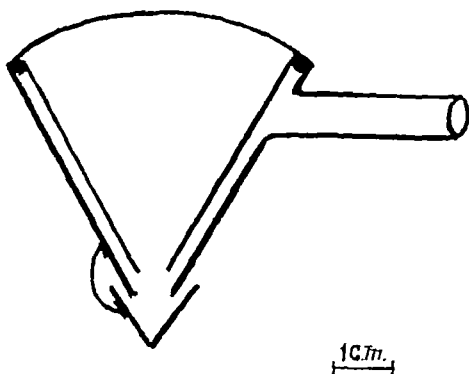
It is found that if individuals breathe to and from a bag (a small air cushion with a mouthpiece fitted is convenient) there is a marked increase in the leucocytes in the peripheral blood, which may number over 15,000 per cu. mm. The number returns to normal, and even below, on over-ventilation. The experiment is convenient for class purposes, as it indicates the ready variability of the leucocyte count. The differential count shows that the increase is chiefly in the polymorph-nuclears.

A convenient method of demonstrating local leucocytosis is to cause hypoxæmia in one hand by hot water and to compare the two sides.



**Drop recorder.** By O. INCHLEY, *King's College, London.*

This consists of a funnel composed of two metal sheets separated by a narrow air-space. The air-space opens below into a small metal cup which when filled with the fluid seals the air-space. The air-space is connected by a side tube with a tambour which is actuated by the impulse derived from the drop falling into the cup.



The figure is actual size and the dimensions are important. It would appear that the cup is not deep enough to seal the air-space, but in fact surface tension insures working as described, and it will work with ether as with water.

**On the correlation between pulse and respiratory tests of schoolboys.** By J. G. WOOLHAM and W. R. HONEYBURN.

**TESTS.** Fifty elementary schoolboys were examined by Air Force tests, the results being combined and handicapped for age in each case. The final result is regarded as an index of each boy's physiological efficiency and is called the Flack-Woolham figure, F.-W. Further, the same boys had the resting pulse counted during three minutes after resting horizontally for five minutes; the standing pulse rate and the exercise pulse rate were counted for a period of six minutes at each test. The exercise given was fifteen steps (up and down = one), stool 18 inches high, duration of exercise 25 seconds. The correlation between pairs from the different sets of tests have been calculated and the following conclusions have been drawn.

**CONCLUSIONS.** The correlation between the resting pulse rate and the F.-W. is negative, also there is a higher negative correlation between the standing pulse rate and the F.-W., i.e. the quicker the pulse rate,

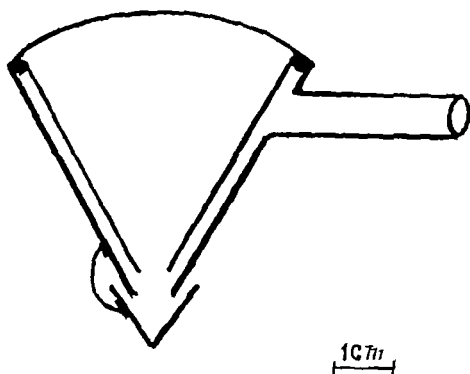
resting or standing, the lower the F.-W. We may say, regarding the figures, that a slow pulse when resting and, especially, when standing is better than a quick rate, but that a slow pulse rate is not a necessity for physiological efficiency. No relation is shown between the pulse rate after exercise (within 15 seconds) and the F.-W. There is a slight positive correlation between the F.-W. and the drop from highest to lowest rate immediately after exercise; in other words, a *deep* plunge is advantageous. A *steep* plunge is advantageous. There is no correlation between the total number of pulse beats counted, subject standing, for six minutes before and after exercise. In one-third of the fifty cases there was a *lower* total, *after* exercise, than before exercise. We find that a large number of beats after exercise is against physiological efficiency, but it appears that a low standing pulse rate before exercise is of better significance than a low pulse rate after exercise when the F.-W. figure is taken as an index of efficiency.

**A method for determining the effect of electrolytes in the lumen of the surviving gut on its movements.** By H. E. MAGEE and B. A. SOUTHGATE. (*Preliminary communication.*)

As it was desired to study the effect of ions on the inside of the surviving intestine, the apparatus sketched was devised. The drawn out ends of the glass tubes *B* and *D* are faced with cycle valve rubber on to which a 3-inch piece of small intestine, *C*, from rabbit or cavy is tied. The gut is kept straightened out in bath, *F*, containing tyrode, for 30 minutes when vigorous contractions will have begun. Fluid, 0.9 p.c. NaCl at 37°, is run into *B*, until there is a hydrostatic pressure of about 6 inches, which is usually excessive. Then *B* is lowered so as to allow for longitudinal contractions. Clips *E* and *A* are opened. Some fluid runs out from *D* and the previously dilated gut contracts from below upwards so that fluid then ceases to flow out. Both clips are closed immediately because dilatation may soon set in. Generally the resulting hydrostatic pressure is approximately the same with pieces of gut from the same animal. The movements are recorded on a slow drum. From time to time 0.1 to 0.5 c.c. of strong solutions of electrolytes are added to *B* so that the hydrostatic pressure is not appreciably affected. It was not possible to keep the volume of fluid constant owing to variations in the tone of the gut from animal to animal. Changes in tone caused variations in the hydrostatic pressure with constant volume. The standard (physiological) pressure, arrived at as above, gave optimum contractions.

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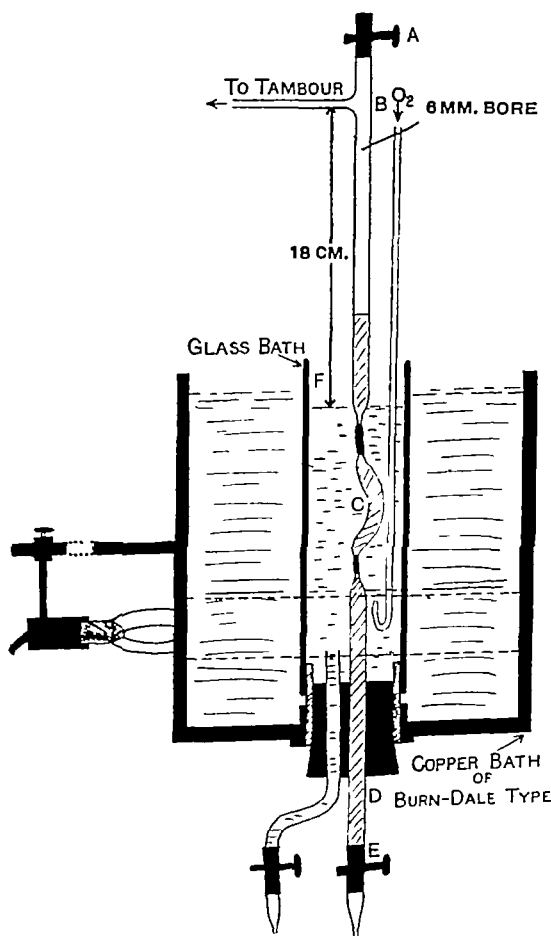
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As, in the first instance, qualitative effects of ions were to be considered, further precautions in regard to the volume of fluid were not taken.

The type of contraction obtained was different from that recorded in the usual way, and varied according to the part of the intestine. Generally the contractions were of a more continuous nature in the most proximal jejunum than in the terminal ileum where they were larger and spasmodic. The ileum was much more resistant to increasing concentrations of  $\text{Ca}$  than the jejunum. Both parts were relatively insensitive to  $\text{PO}_4$ , but the ileum more so than the jejunum.



# PROCEEDINGS

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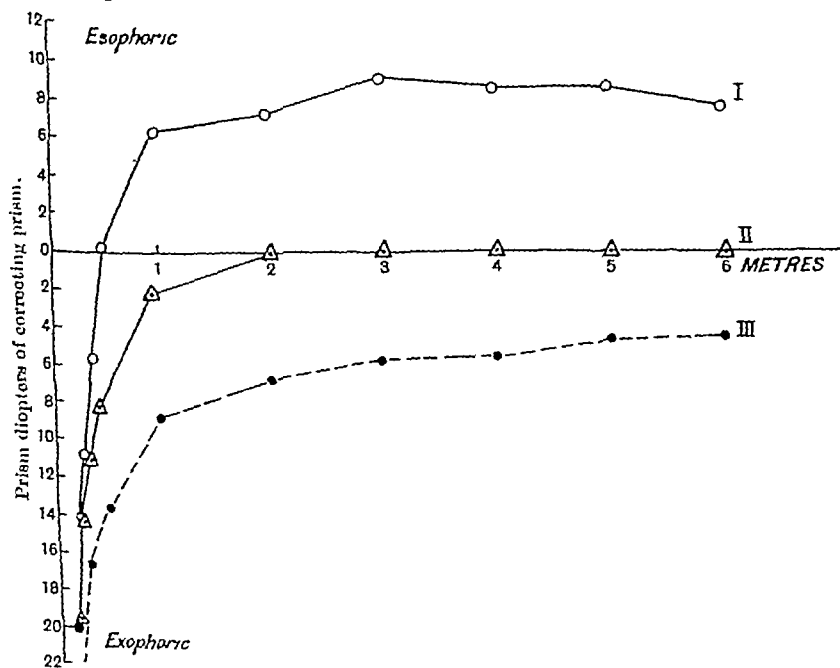
# PHYSIOLOGICAL SOCIETY,

### March 17, 1928.

#### Balance of ocular muscles in normal subjects.

By A. N. BIRKETT and F. W. LAMB.

All the subjects had a visual acuity of  $\frac{6}{6}$  Snellen. The images in the two eyes were dissociated by a Maddox rod and the "phoria" measured by a rotatory prism in front of the other eye. The test object was a circular aperture, illuminated from behind, in a dark room.



I Esophoric. II Orthophoric. III Exophoric Subject.

The distance of this object from the subject was varied from 6 m. to 20 cm. the aperture being diminished for the nearer positions. The average of a number of readings at each distance was taken and the results graphed: the distance of the object on the abscissa and the prism diopters of the correcting prism on the ordinate so that esophoric corrections fall above the abscissa and exophoric below. Orthophoric cases,

in which no correction was required, would therefore fall on the abscissa (Figure). Since all subjects are exophoric at short distances from the test object, the graph of those who are esophoric at greater distances must cross the abscissa when the subjects become orthophoric. This transition or orthophoric point varied from about 30 to 50 cm. distance. The orthophoric subjects remained orthophoric from the nearest orthophoric point up to 6 m. The exophoric subjects never became orthophoric, *i.e.* their graph remained below the abscissa at all distances. Most of the subjects gave a graph which, beyond the orthophoric point or in the case of exophoria beyond 1 m., ran parallel to the abscissa. This also held good when series of tests were carried out on a subject at different times during a day, though these graphs did not always lie at the same distance from the abscissa, that is, diurnal variations occurred. In other subjects this parallelism was absent, the graphs for different hours sometimes crossing and showing irregular variations from one testing distance to another: possibly evidence of muscular instability.

The percentage distribution found was: exophoric 17 p.c., esophoric 77 p.c. and orthophoric 6 p.c. These percentages are in harmony with those reported by Thomson for school children<sup>1</sup>.

It would seem that the statement which is so often made that orthophoria is the normal condition is incorrect.

### **Posture deviations of the arm and their reversal.** By F. W. LAMB, E. D. PORTMAN and G. J. WOOLHAM.

The deviations which occur when the arm of a normal subject is held in different positions and the changes induced when the positions of the other limbs or of the head are altered or when the labyrinths are stimulated, have been recorded by means of a "Posture Board" which analyses the movements of the arm into two components in the horizontal plane, namely, "right and left" and "back and forward."

The principal results were as follows:

(a) The arm was held in a forward position similar to that assumed in piano playing. The deviations were constant in direction in a given individual but were not constant from subject to subject; the majority (13 out of 19 subjects) gave a "back and left" response, *i.e.* towards the resting posture of the arm. This tendency to deviate to the position of rest or of least strain was more marked when the arm was adducted, *i.e.* across the subject's body (12 out of 14 subjects), and when abducted (13 out of 14 subjects). Voluntary rigidity in the arm muscles increased the

<sup>1</sup> *Brit. Journ. Ophth.* 9, p. 109. 1925.

rate of development of these deviations. Fatigue of the arm by weight holding previous to recording caused more rapid deviations without alteration in direction.

(b) Phenomena of Reversal. The influence of different positions of the head, of stimulation of the labyrinth, of eye movements and of position of the legs on type of deviations in the piano-playing position of the arm, gave in general the following results:

1. Lateral inclination of the head—right or left. A definite reversal or a tendency thereto occurred either between the deviations in the two inclined positions or between the “normal” upright head position and one or other of the inclinations. No uniformity was found between the direction of head inclination and the accompanying deviation.

2. Flexion and extension of the head. The effects were generally slight but in the more marked cases extension of the head caused a forward deviation of the arm (extension) and head flexion gave flexion of the arm.

3. Rotation of the head. The effects were more marked than with head inclinations. A reversal or a tendency thereto occurred in the majority of cases. Where no reversal occurred, the change was either in the degree or in the rapidity of the deviations.

In general there was evidence that the arm tended to deviate towards the opposite side from that to which the chin points.

4. Position of the opposite arm. When the opposite arm, *e.g.* left, was raised to shoulder level and abducted, the deviation of the right arm was to the right, *i.e.* a balance deviation. Holding a weight in the left hand in this position made the deviation of the right more pronounced.

5. Position of the lower limbs. Extension and abduction of the leg (*e.g.* left) gave definite deviation of the right arm to the right. A similar position of the right leg gave a left deviation in the right arm.

6. Labyrinthine stimulation with water below body temperature. All cases gave similar results. Stimulation of the right ear gave a deviation to the left in the right hand, and stimulation of the left ear a deviation to the right. Therefore stimulation of an ear caused both arms to deviate in the same direction, *e.g.* right ear stimulation causes both hands to deviate to the left.

7. Eye movements. When the hand was screened from sight, evidence was obtained that the hands tended to deviate in the direction of the lateral eye movements.

8. Experiments on the left arm. These confirmed those on the right. No definite evidence of asymmetry of responses on the two sides was obtained.



**A simple method for the ultrafiltration of undiluted blood serum**By FRANK CAMPBELL SMITH (*London Hospital Medical College*).

For the purposes of another investigation it was necessary to devise a method for the ultrafiltration of undiluted blood serum sufficiently rapid and simple to deal with two or three sera a day.

The method to be described was evolved as the result of the following considerations:

(1) Quite a low pressure—say 65 mm.—should be sufficient, since as soon as the filter becomes blocked, the pressure will have very little influence on the rate of filtration.

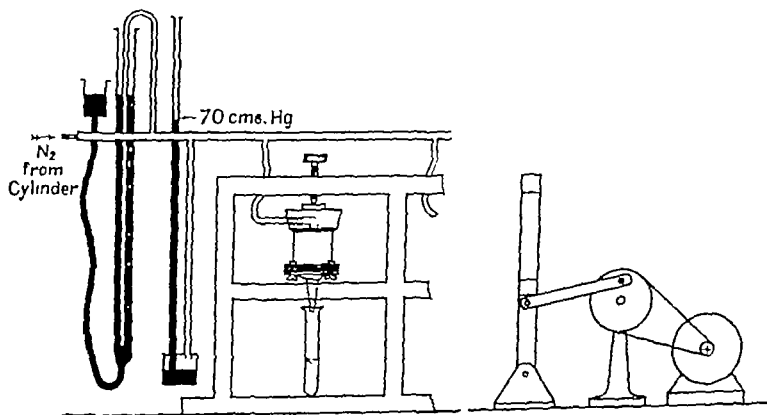
(2) The larger the area of membrane used, the greater should be the rate of filtration.

(3) In a solution of protein as concentrated as serum, a fairly permeable membrane, which allows some protein to pass, should allow of a fairly rapid first filtration. The resulting filtrate, now of a much lower concentration, should present little difficulty in being entirely cleared of protein by a second and less permeable membrane.

(4) A means of agitation should be employed, thereby increasing the rate of filtration, as pointed out by Pierce (December, 1927).

*Method.*

*The filter.* Seitz's filters, 6 cm. diameter for the first filtration and 3 cm. for the second, were used. The asbestos disc was replaced by the



collodion membrane, a piece of filter paper being placed between a layer of silver gauze and the membrane. The filter designed for negative pressure was used, as this type is much more convenient for setting up the membrane. It is important to use those supplied with three vertical

pins which maintain the position of the upper part of the filter, while the two sections are clamped together by means of butterfly nuts. The method by which a positive pressure was applied to this filter is clearly shown in the figure.

*Collodion membranes.* The coarse membranes were made from a 3 p.c. solution of pyroxylin (B.D.H.), dissolved in 75 parts alcohol, 25 parts ether, and 10 parts glacial acetic acid. The finer membranes were made from a 3 p.c. solution in 50 parts alcohol, 50 parts ether, and 10 parts of glacial acetic acid.

The solution was poured on a perfectly clean photographic quarter-plate, which was supported on a brass table, 8 inches square. This table was provided with two very sensitive spirit levels at right angles, and had an adjustable three-point suspension. The drying was allowed to continue until ether could no longer be smelt, and up to the point when a reflection on the surface, such as an electric-light bulb, just lost its sharp definition. The glass plate was then placed in water, when the membrane usually floated off the plate by itself.

Membranes made by this somewhat crude method are naturally rather unreliable as to their permeability, but have served the purpose for which they were made.

The ethylene glycol membrane described by Pierce<sup>1</sup> is being tried at present and it is hoped that better grading of permeability will be obtained.

Movement of the serum at the surface of the membrane was brought about by introducing a few glass beads and by rocking the frame supporting the filter from side to side through an angle of about 30° from the vertical.

By carrying out the method as indicated above, 5-6 c.c. of protein-free filtrate should be obtained from 10 c.c. of serum within 6 hours. The second filtration has usually taken not more than 20 minutes, and if the first be left overnight, little time is lost.

### **The action of pituitary extracts upon isolated blood-vessels.**

By E. D. PORTMAN and A. D. MACDONALD. (*Manchester*.)

The diuresis provoked by intravenous injections of extracts of the posterior lobe of the pituitary body in the anæsthetised or decerebrate cat is associated with kidney dilatation. In the study of this volume change much has been made of the dilator action of the extracts on the

<sup>1</sup> Pierce, H. F. Nitrocellulose membranes of graded permeability. *Journ. of Biol. Chem.* 75. p. 795. 3 Dec. 1927.

renal artery(1, 2) while on all other arteries tested varying degrees of constriction are obtained. While such relaxation would not contribute significantly to the plethysmographic changes directly, it might increase the blood supply to the kidney. In the perfused kidney, it must be remembered, pituitary extracts proved constrictor and antidiuretic (Dale(3)).

We have used chains of six rings cut from arteries and veins of the cat, rabbit, dog and sheep, and have found that purified posterior lobe extracts (*i.e.* free from the depressor or histamine-like substance), though very rich in both pressor and oxytocic principles and though producing marked kidney dilatation and a copious diuresis in the cat, are without action alike upon the carotid or femoral and renal arteries and veins, even using concentrations as high as 0.05 p.c. of dried gland. The renal vessels were investigated throughout their lengths, including their first branches. That the absence of response to such extracts is not due to over-weighting or a lack of sensitivity of the preparations is proved by their free contraction to such a stimulus as adrenaline or histamine in a concentration of one in five to ten millions. We are at a loss to explain the dilatation of the renal artery described by earlier observers, unless it be due to the use of commercial or acid extracts.

1. Sharpey-Schafer. *The Endocrine Organs*. 2nd ed. Pt. 2.

2. Cow. *J. Physiol.* 42, p. 134. 1911.

3. Dale. *Biochem. J.* 4, p. 427. 1909.

### **Assessment of schoolboys by air force tests.**

By F. W. LAMB and J. V. A. SIMPSON.

Assessments were made on the following groups of boys attending elementary schools, ages ranging from 10 to 16.

I. A sample, selected for a course of three months' training in rowing. 23 subjects.

II. A similar sample of 9 who did not carry out continuous training.

III. A group of 10 who acted as coxswains to I and II.

IV. A sample from an open-air school of boys who were definitely below normal.

V. A random sample from the school population.

The assessments were:

1. The Woolham modification of the Flack product, based on the maximal expiratory pressure, the time for simple breath holding and the time for persistence test at half the maximal expiratory pressure with a correction factor for age.

2. The types of pulse response to the persistence test.
3. The changes in the systolic blood pressure during the persistence test.

4. The weight and height expressed as the Pedilisi index.

The main conclusions arrived at were:

1. The three types of pulse response described by Flack in adults occurred amongst the boys, viz. (a) the sustained, (b) the slow continuous fall and (c) the unstable or a rapid initial rise followed by an early sharp fall.

The type was constant for a given subject on different occasions, not too far apart in time.

2. Corresponding to these pulse types, three general types of systolic pressure changes, (a) a small rise with slight variations around the maximal point, (b) a gradual rise throughout the period accompanied by a gradual fall in rate, (c) a marked rise and fall with an unstable pulse.

3. A low Flack-Woolham product was in general associated with the unstable type of pulse; a high value with a well-sustained type.

4. There was no relation found between the anatomical assessment (Pedilisi) and the functional assessments.

5. The effects of training on sample I were shown when the assessments made after the training were compared with those before and with those of the other samples.

(a) More moderate rises in heart rate; improvement in sustaining the rate and fewer cases of the unstable types.

(b) Changes in the Flack-Woolham product.

	1st assessments			2nd assessments		
	Highest	Lowest	Average	Highest	Lowest	Average
Sample I (training)	0.77	0.15	0.38	1.38	0.16	0.61
„ II (less training)	0.52	0.16	0.3	0.63	0.26	0.39
„ III (coxswains)	0.49	0.14	0.29	0.64	0.16	0.31
„ IV (open-air school)	0.42	0.03	0.19	—	—	—
„ V (random)	0.73	0.13	0.33	—	—	—

Note.—A range of 0.4 to 0.5 may be taken to be about the normal range for elementary schoolboys (Woolham).

### The tension of oxygen in human urine. By G. A. BUCKMASTER and H. R. B. HICKMAN.

Both human urine and ox bile always contain small amounts of oxygen. This is probably in physical solution. There is therefore a definite pressure of this gas in these liquids; this varies but the mean oxygen tension is of the order of 60 mm. Hg<sup>1</sup>.

<sup>1</sup> Buckmaster and Hickman. Proc Physiol. Soc. Journ. Physiol. 61. 2. 1926.

Direct determinations of the tension of oxygen in human urine have been made to see how far these are in agreement with the above result.

About 200 c.c. of urine, direct from the bladder, were taken at the same time of day and equilibrated at  $37.5^{\circ}$  C. in specially devised tonometers for two hours with known mixtures of oxygen and nitrogen. The gas volume was about 50 c.c.

After an experiment, the transfer of gas to a Haldane's gas analysis apparatus was accomplished so that no temperature change occurred during the transference.

Carbon dioxide tensions were measured at the same time, and experiments show that the pressure of this gas in urine is about 45 mm. Hg.

The following values taken from three times the number of actual experiments, show that in many cases the pressure of oxygen in urine must be between 50 and 60 mm. Hg; it has never been found to be less than 39.5 mm.

#### *Determination of Oxygen tension in Urine.*

Initial tension in mm. Hg .	60.30	52.50	50.86	38.00	39.25
Final tension in mm. Hg .	67.04	59.54	62.32	39.50	40.27

PROCEEDINGS  
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**Oestrus and pseudopregnancy in the ferret.** By J. HAMMOND  
and F. H. A. MARSHALL.

The ferret has a well-marked breeding season lasting from about the middle of March to the middle of August; from September to February it is in an anoestrous condition.

As the ferret does not ovulate spontaneously, but only after coitus or the orgasm resulting from this, it remains on heat continuously throughout the breeding season if unmated (Nos. 1 and 8). A series of animals mated at different times during the breeding season showed that ovulation can occur at any time during this period, *i.e.* ripe follicles are always present in the ovaries (of unmated animals) from about March to August; in unmated animals there is therefore no cycle.

If females are mated with sterile (vasectomized) males ovulation occurs and a cycle (or pseudopregnancy, lasting for about 6 weeks followed by a return of heat at 8 weeks) is introduced (No. 9). The cycle can thus be produced by sterile matings at any time desired during the breeding season and does not depend on any innate rhythmical process apart from ovulation and the formation of corpora lutea.

The onset of heat is marked by a swelling of the vulva which usually reaches maximal size in about 2-3 weeks (Nos. 1 and 3), when mature follicles are present in the ovaries, and the animal remains in this state throughout the whole of the breeding season. This outward sign (the swelling is about 50 times the size of the anoestrous vulva and can be measured fairly accurately by tracings on glass) of the production of oestrous hormone we consider is due to the presence of ripe follicles in the ovaries since it is absent during anoestrus, when only small follicles are present (Nos. 4, 8 and 9), and is absent during pregnancy and pseudopregnancy (Nos. 2, 5, 6 and 7), when corpora lutea and small follicles only are present.

The uterus is very small and the glands are comparatively undeveloped during anoestrus (Nos. 4, 8, 9). With the onset of oestrus the

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urethra was never constricted in any way, but in all cases the skin at the meatus was drawn forwards and closed carefully by clips. The bladder was not greatly distended, the pressure, with the gas present, being about 7-10 mm. Hg as measured by manometer.

There was a general resemblance between the gas tensions in the bladder and those in gas in the abdominal cavity of the same animals. On some few occasions the CO<sub>2</sub>-tensions in the bladder were distinctly lower than those in the abdominal cavity, whilst at the same time the

Table I.  
*Gas tensions.*

Animal	CO <sub>2</sub> -tensions; mm. Hg			O <sub>2</sub> -tensions; mm. Hg		
	Under skin	In abd. cav.	In bladder	Under skin	In abd. cav.	In bladder
Rabbit 1	53	52	49 (a) 40 (b) 43 (c)	20	38	45 34 48
2	46	49	53 (a) 63 (b)	14	37	33 36
3	46	50	50 (a) 43 (b)	28	36	39 53
4	57	56	56 (a) 48 (b)	14	34	38 34
5	51	51	37	15	35	45
6	54	57	40	17	34	45
7	53	54	42 (a) 41 (b)	21	35	61 41
8	49	48	46	25	39	40
9	49	47	42	21	35	41
10	48	48	46	21	44	46
Monkey	40-49*	39-49*	47	35-46*	38-47*	42

\* Figures obtained from 5 other monkeys. (a), (b), (c) separate experiments.

O<sub>2</sub>-tensions were higher; this is possibly due to temporary hyperæmia in the bladder. It has been shown (1) that during the first few days after injection of gas under the skin and into the abdominal cavity that the CO<sub>2</sub>-tensions are lower and the O<sub>2</sub>-tensions are higher than they are later when the tissues have become accustomed to the presence of the gas; the tensions given in the Table for the skin and the abdominal cavity were obtained after the tissues had become accustomed to the gas; of course it was not possible to keep the gas so long in the bladder, and in any case the bladder would not be so disturbed by the injection because there is a cavity there already, accustomed to being distended.

We may conclude that the CO<sub>2</sub>-tensions in the cavity of the bladder usually lie between 40-50 mm. Hg, whilst the limits for O<sub>2</sub>-tensions are usually 35-45 mm. Hg. As a rule there was little urine in the bladder, but even when a large quantity was present at the end of the three



uterus enlarges and the mucosa and glands are rather better developed, but, as with the vulva, after the maximal size is reached no further development occurs however long the animal may remain on heat. In pseudopregnancy on the other hand the uterus increases greatly in size and in the development of the glands, the increase proceeding up to the 5th-6th week (or about the same time as the normal duration of pregnancy). At about the 6th-7th week the enlarged glands (gland cells and their nuclei, which become greatly hypertrophied and are similar to the giant cells of the placenta) are cast off into the lumen of the uterus and breakdown occurs.

In virgins the mammary glands are undeveloped both in anæstrum (No. 4) and also during œstrus. However long the animal may remain on heat no difference in the development of the mammary gland is apparent (Nos. 1 and 8). During pseudopregnancy the ducts of the mammary glands grow and the ends enlarge forming a thick mass (about the size of a shilling) surrounding the nipple, which itself also increases greatly in size during pseudopregnancy (Nos. 2, 5, 6, 7). In an animal which has previously been pregnant (No. 3) or which has had a pseudopregnant period during the breeding season (No. 9) and has come into œstrus or gone into anæstrum the mammary glands maintain the duct length which has been previously developed, but the ducts become very attenuated and the nodular alveolar growth disappears presenting a very atrophic appearance. There can be no doubt that the corpus luteum not the follicle or œstrous hormone is responsible for mammary and development<sup>1</sup>.

#### **CO<sub>2</sub>- and O<sub>2</sub>-tensions in the bladder.** By J. ARGYLL CAMPBELL.

Using the bladder (male rabbits, monkey) as a tonometer, about 10-25 c.c. of gas containing known percentages (usually 5-6) of CO<sub>2</sub> and O<sub>2</sub> were injected by means of a catheter (ordinary technique) and the gas was left there for periods up to three hours. At the time of injection any urine in the bladder was drawn off, the catheter being withdrawn immediately after the injection of gas, and used again when samples of gas were required for analysis, care being taken to exclude air. The animals used were accustomed to being fixed on their backs; with careful attention to their comfort and with their eyes covered they kept very quiet and often appeared to sleep. To retain the gas the urethra was sometimes partially blocked by a short length (5 mm.) of glass rod; the

<sup>1</sup> The numbers refer to specimens shown at the meeting.

By analysis of cross-test results obtained on material of known potency it was found that the following animals were liable to cause inaccuracies:

(1) Rabbits that had been used on rough tests and had therefore received doses of insulin varying widely from  $\cdot 5$  unit per kg., which is our standard dose.

(2) Animals below 1600 and above 3000 grm. in weight.

(3) Animals that have been in use for more than one year.

(4) Females that had been used for breeding.

(5) "Ginger" rabbits and Belgian hares.

The method of basing the dose of insulin on the weight of the animal appeared to be satisfactory between the limits of 1600 and 3000 grm. Grouping the results from about 1700 injections according to the weights of the animals gave a mean value of 26.2 for the percentage reduction of those under 1600 grm. and of 37.3 for those over 3000 grm., but all other weight groups showed means which did not differ significantly from 29.0 p.c. On the other hand weight has an effect on the third type of variability as shown by the fact that inaccurate results are obtained in cross-tests if there is not a good balance of weight between the two groups.

An analysis of the results for different seasons of the year showed that they lay on a fairly regular curve with a maximum mean percentage reduction of 32.8 in June and a minimum of 24.1 in December.

That seasonal variations might mask those due to weight was suggested by calculation of the average weights of the animals used at different times of the year. As a minimum was found in the summer and a maximum in the winter this possibility cannot be excluded.

A method has been evolved for the calculation from a cross-test (without assuming its result) of a "day variation factor" (*i.e.* the ratio of the total percentage reduction on one day to that on a subsequent occasion, the animals receiving the same dose) for groups of rabbits and its magnitude examined. For accurate results this factor should not differ from unity by more than  $\cdot 2$ .

The calculation of the day variation factor has been applied to the examination of a method for insulin assay (used for some time for rough tests in this laboratory) in which the cross-test principles are observed but which admits of a larger number of results for the same amount of labour (with some loss of accuracy, the average error being 11 p.c.).

hours' time, the tensions were not obviously changed in the gas in the bladder.

If the gas tensions in urine differ from those in the bladder, the longer the urine is in the bladder the closer should be the approximation to bladder tensions. The  $O_2$ -tensions in seven samples of human urine from a bladder not emptied for twelve hours (over-night concentrated urine) were found to lie between 14–25 mm. Hg; and the  $CO_2$ -tensions between 40–50 mm. Hg; in a sample of very dilute urine (Sp. G. 1000) formed rapidly (45 minutes) in water diuresis, the  $O_2$ -tension was 35 mm. Hg and the  $CO_2$ -tension 43 mm. The gas tensions in the urine were estimated by a tonometer method, similar to that of Buckmaster and Hickman(2), but all the manipulations were carried out in a hot room (37.5° C.). The  $CO_2$ -tensions in human urine resembled those in the bladders of the animals given in the Table, but the  $O_2$ -tensions were often distinctly lower in the human urine examined. Krogh(3) and others obtained similar low figures for  $O_2$ -tensions in human urine, but some observers(2) obtained higher results. The factors controlling  $O_2$ -tensions in urine require further elucidation.

(1) Campbell. *This Journ.* 59. p. 1. 1924; 61. p. 248. 1926, etc.

(2) Buckmaster and Hickman. *Proc. Physiol. Soc.* March 17th, 1928.

(3) Krogh. "Respiratory Exchange of Animals and Man," p. 77. 1916.

### **Some factors affecting the response of rabbits towards insulin.**

By KATHLEEN CULHANE and S. W. F. UNDERHILL. (*From the Physiological Laboratories, The British Drug Houses, Ltd.*)

In the assay of insulin by the determination of the reduction of the blood sugar of normal rabbits three types of variation in response are liable to occur:

(1) Variability in the degree to which the blood sugar is reduced by the same dose of insulin as measured by the average fall in five hours expressed as a percentage of the initial value. The average percentage reduction following a dose of .5 unit per kg. was found to be 29.0 with a coefficient of variability of 36.6 p.c.

(2) Variability in the percentage reduction in the same animal when given the same dose on different occasions.

(3) Variability of groups of animals apparently due to a change in sensitiveness which affects the whole stock at the same time.

Notwithstanding these variations it is possible by careful selection of animals to obtain results by Marks'(1) cross-test method showing errors of less than 5 p.c.

By analysis of cross-test results obtained on material of known potency it was found that the following animals were liable to cause inaccuracies:

(1) Rabbits that had been used on rough tests and had therefore received doses of insulin varying widely from  $\cdot 5$  unit per kg., which is our standard dose.

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**Effect of adrenaline, sympathol, tyramine, ephetone and histamine on gas exchange and circulation in man.** By U. v. EULER and G. LILJESTRAND. (*From the Department of Pharmacology, Caroline Institute, Stockholm.*)

In a trained human subject the effects of adrenaline, sympathol, tyramine, ephetone, and histamine on oxygen consumption, pulse rate, systolic and diastolic blood-pressure, and minute volume of the heart were determined. The pulse pressure, divided through the mean arterial pressure ("reduced pulse pressure") multiplied with the pulse rate, gives a simple method of obtaining an approximate idea of the blood flow<sup>1</sup>. For the sake of comparison also the optimum concentration, in which the substances gave acceleration of the reduction rate of methylene blue, was determined. The following table gives the main results. If the effect is less than 5 p.c., it has been put down as 0. With + a rise above the normal value of 5-10 p.c., with ++ a rise of 10-30 p.c. and with +++ a rise above 30 p.c. is meant.

Substance	Dose in gm.	Maximal effect on					Optimal concentration on red of methylene blue
		Pulse rate	Mean art. press.	Oxygen cons.	Pulse rate $\times$ red pulse press.	Minute volume of the heart	
l-Adrenaline*	0.0007	+	+	++	+++	+++	10 <sup>-12</sup>
d-Adrenaline	0.0007	+	0	+	+++	+++	10 <sup>-10</sup>
Sympathol	0.05-0.1	+	+	+	++	++	10 <sup>-4</sup>
Tyramine-phosphate	0.02-0.05	+	+	+	+++	+++	10 <sup>-5</sup>
Ephetone	0.05	++	0	+	+++	+++	10 <sup>-6</sup>
Histamine-phosphate	0.0004-0.0007	+++	+	+	+++	+++	10 <sup>-11</sup>

<sup>1</sup> Cp. G. Liljestrand and E. Zander, *Zeitschr. f. d. ges. exp. Med.* vol. LIX, p. 105, 1928.

<sup>2</sup> From U. v. Euler and G. Liljestrand, *Skand. Arch. f. Physiol.* vol. LII, p. 243, 1927.

**Mucus secretion of the colon.** By H. FLOREY and A. N. DRURY\*. (*Preliminary communication.*)

The following results have been obtained during an investigation of the adequate stimuli for mucus secretion from the colon. For acute experiments cats were used.

It was found that electrical stimulation of the sacral nerve roots posterior to, *i.e.* not including, the 1st sacral, after a suitable exposure in the spinal canal, resulted in a copious secretion of thick mucus in the colon. Stimulation was carried out for 4 hours by means of faradic shocks thrown into the nerve for  $\frac{1}{2}$  sec. every 2 sec. (approx.). Pilocarpine

\* Working on behalf of the Medical Research Council.

1/1000 applied locally to loops of colon produced in from  $\frac{1}{2}$  to 1 hour a copious secretion of mucus. This was inhibited by the previous local application of 1 p.c. atropine sulphate. Mustard oil, diluted with olive oil, applied locally, produced a copious secretion of mucus in  $\frac{1}{2}$  to 1 hour. This reaction however was not inhibited by previous thorough atropinisation.

For experiments of a more extended character the following preparation of a dog has been made. Under ether and morphia a suitable portion of colon was cut to form fistulae subsequently, and the continuity of the colon restored by end-to-end anastomosis. The portion cut out, still retaining its blood supply, was then divided into two pieces, the upper ends of which were insewn and the lower brought as fistulous openings to the skin. These healed in perfectly and gave a preparation with two small separate pouches of colon.

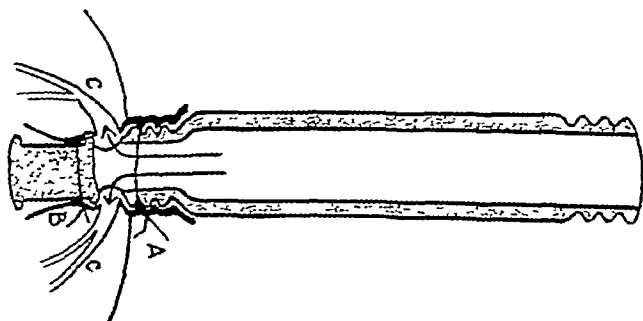
Histological examination of the mucosa lining such pouches shows that after 3 months it is indistinguishable from that lining normal colon.

#### A modified cannula for perfusion of the isolated heart.

By R. RÖSSLER (Rockefeller Travelling Fellow).

(From the Physiological Laboratory, Cambridge.)

For accurate registration of the coronary blood flow in isolated perfused hearts it is important to obviate the leak of fluid through the aortic valves. The leak which may be as big as 40 per cent. of the total flow



Cannula for perfusion of the isolated rabbit's heart. The cannula is made of metal. Ligatures *A* above and *B* below the coronary arteries, *C* the coronary arteries.

is not constant for any given heart but varies with temperature, changes in the heart rate and perfusion pressure (Sharpey-Schäfer, Wiggers). Sharpey-Schäfer obviated the leak by pushing the perfusion cannula

beyond the valves. This allowed the fluid to distend the left ventricle and pass along the outside of the cannula into the coronary arteries, its escape through the pulmonary veins being prevented by a ligature. The disadvantages of this method are (a) the ventricle is over-distended, (b) the perfusion pressure varies with changes in the strength and the rate of contraction. Introduction of cannulæ directly into the coronary arteries does not prove practicable, since in most hearts the main division of the coronary arteries is too close to their origin, and moreover the cannulæ considerably increase the resistance to the flow and the movement of the heart is liable to occlude the orifice of the cannula at one or another phase of the cycles. For accurate registration of the coronary circulation (especially by the hot wire method) a cannula had to be devised which would (a) obviate any possible leak through the valves, (b) introduce no significant additional resistance, (c) render impossible any obstruction of its orifice by the movement of the beating heart. The problem was solved as shown in the figure. A fine ligature is passed underneath both coronary arteries which is easy if the artery is slightly lifted up by a seeker introduced from the aorta into the orifice of the artery. The aortic cannula is then introduced so that its lower end protrudes below the ligature. The ligature is then tied just below the flange of the cannula so that the orifices of the coronary arteries are above the ligature; a second ligature is now tied in the groove a little above the orifices of the arteries. The space between the ligature is filled through the slots of the cannula by the perfusion fluid which can escape only through the arteries. Clamping of both arteries stops the flow, showing that there is no leak whatever; the bore of the cannula and of its slots is considerably bigger than that of both arteries, no extra resistance to the flow is, therefore, introduced; contraction of the heart cannot of course obstruct the orifices of the cannula nor change the perfusion pressure. Hearts perfused by this method beat as well as in the case of the ordinary aortic perfusion; they beat considerably better than in the case of introduction of cannulæ directly into the coronary arteries.

**The effect of cold on the adrenaline content of the suprarenal glands.** By G. P. CROWDEN and M. G. PEARSON. (*Preliminary communication.*)

The following experiments show that when cold produces a marked fall in the body temperature of the cat there is considerable depletion of the adrenaline content of the suprarenal gland with normal innervation as compared with the denervated gland.

*Method.* The splanchnic nerves and nerves to the semi-lunar ganglion of the left side were cut by ligature. The animal was then exposed to cold in a room at 0° C. for periods of 1½ to 8 hours. The animal was then killed, the glands removed and their content of adrenaline estimated by the colorimetric method of Folin, Cannon and Denis.

	Operative Procedure	Duration of cold	Fall in body temperature (rectal)	Depletion of innervated gland. (Content of denervated gland 100 p.c.)
Exp. 1. Cat ♂	Left splanchnic nerves and nerves to left semi-lunar ganglion cut. Animal not fed in morning	4½ hrs.	39.3-34 = 5.3° C.	29 p.c.
Exp. 2. Cat ♂	ditto.	4 hrs.	38.4-33.9 = 4.5° C.	33.5 p.c.
Exp. 3. Cat ♂	ditto.	1½ hrs.	37-25 = 12° C.	14.5 p.c.
Exp. 4. Cat ♂	Left nerves cut. Animal fed in morning	8 hrs.	38-37.5 = .5° C.	Nil
Exp. 5. Cat ♂	Left nerves cut. Animal not fed	7½ hrs.	38.4-37 = 1.4° C.	Nil
Exp. 6. Cat ♂	Ligature left in position, nerves not cut. Animal not fed	Nil	Constant	Nil

The expenses of this research have been defrayed by grants from the British Medical Association and the Medical Research Council.









PROCEEDINGS  
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**The total depolarisation of crustacean nerve.**

By K. FURUSAWA.

In 1926, shortly before his sudden and untimely death, Levin described the remarkable behaviour of crustacean nerve in response to tetanic stimulation. The action potential appears to persist for a considerable period after the stimulus. This phenomenon, which he termed the "retention of action current," is very striking yet easily observable with crustacean nerve. Levin found also a "ceiling effect," which signifies that under favourable conditions the maximal galvanometer deflection caused by a group of tetanic stimuli reaches a constant level: and he recognised the profound local fatigue which arises near the stimulating electrodes.

The first clue to an explanation of this peculiar phenomenon was provided by a number of observations on the injury current and the maximal galvanometer deflection, employing a single pair of stimulating electrodes. Frequently these two quantities were connected by a linear relation or by a simple parabolic proportionality. The injury current falls off gradually after dissection and the maximal galvanometer deflection falls also. There is, moreover, a strong indication that injury and action potentials vanish together. This latter observation suggests that the two quantities are not independent but related to one another.

The "ceiling effect" provides a second clue. Two tetanic stimuli are applied to a nerve. As soon as the first one gives a maximal deflection,  $D_1$ , the stimulus is stopped and the potential divider which supplies the current to balance the injury potential is adjusted to the value ( $P_2$ ) required to bring the spot of light to zero. The second stimulus is then applied, the result being a deflection,  $D_2$ . The ratio  $\frac{D_1 - D_2}{P_1 - P_2}$ , where  $P_1$  denotes the initial injury potential, gives the decrease of deflection (say in mm.) accompanying a drop of one unit (say 1 millivolt) in the injury potential. This value was compared with one similarly obtained by the



**The antirachitic effect of ergot.** By E. MELLANBY, E. M. SURIE  
AND D. C. HARRISON.

In the course of feeding experiments in which ergot of rye was added to the diet, it was noticed, although the experiments were carried out for quite another object, that this substance had a definite, and, in the case of some specimens, a powerful antirachitic action. It is difficult to get young dogs to eat more than 2 to 4 grm. of ergot daily because of its general toxic effect, but even in these quantities its power to promote calcification is striking. The general trend of recent work on the subject of the calcification of bones has been to emphasise the fact that calcification, whether specifically induced by food or by ergosterol after irradiation by ultra-violet rays, is due to one factor, the antirachitic vitamin first described by one of us in 1918(1). It seemed most probable, therefore, that the antirachitic action of ergot was due to the presence in it of vitamin D. This was of particular interest because it will be remembered that ergot was the substance from which ergosterol was first isolated by Tanret(2) in 1889. It is possible that Tanret actually obtained vitamin D with the ergosterol.

Up to the present time, however, it has not been possible to prove definitely that the antirachitic substance in ergot is vitamin D. Like vitamin D it is soluble in alcohol and can be removed by it from ergot. On evaporating off the alcohol the active substance can be dissolved in ether, leaving an insoluble resin, free from calcifying influence. If, after removal of the ether, the fatty residue be saponified by alcoholic potash and extracted with petrol ether, then the active substance, if it is vitamin D, ought to be in the extract together with the other unsaponifiable substances. Ergosterol is present, but so far most of the calcifying factor has been left in the soap, traces only being associated with the inactive ergosterol. It is hoped by using other solvents to separate this factor entirely from the soap.

If the calcifying action of ergot is due, as seems most probable, to vitamin D the question must be faced as to its mode of production. It is possible that it may have its origin in the action of sunlight on the ergosterol of the developing ergot. On the other hand, ergot has a bluish-black covering which is probably impenetrable to radiations, and this suggests that the active factor may be produced directly from ergosterol in ordinary growth independently of sunlight.

1 Mellanby, E. *This Journ. Proc* 52 pp xi and lxx. 1918.

2 Tanret. *Compt rend. Acad. Sci.* 108 98. 1889.

external application of one unit of potential difference when the nerve was in a resting condition. The result showed a remarkable agreement between the two sets of observations.

A third step in the analysis was then taken. A wax chamber with three pairs of filter paper stimulating electrodes was made. These electrodes are to be used in succession: thus as soon as the first pair of electrodes gives a maximal deflection the stimulating current is switched over to the second pair, and so on. Three pairs were found sufficient for the present purpose. The injury potential was not balanced, so that the galvanometer, suitably shunted, registered a deflection due to the injury current. Under favourable conditions (*i.e.* if the nerve had the same potential along its length and if the injury at the end were complete) the spot of light from the galvanometer came back exactly to its zero as the result of stimulation. This means that the injury potential is abolished quantitatively by the action potential. It has often been observed that an injury potential as high as 10 millivolts or more can be abolished completely in this way. The maximal effect is reached about 5 seconds from the beginning of stimulation. If we assume that in the injury potential we are dealing with a polarisation potential across the surface of the nerve fibre (*i.e.* that a galvanometer lead at the injured end is connected essentially to the inside charge, and the lead on the intact nerve to the outside charge, of a certain membrane at which the potential difference resides), then the result obtained is nothing but a total depolarisation of the nerve fibre. In other words the nerve fibre is completely discharged by a tetanic stimulus, and the gradual return of the injury potential (described by Levin as an abolition of the "retention of action current") is really a re-charging of the surface of the nerve fibres.

In nitrogen the injury potential falls off quickly and reaches a low level from which it can be restored to its original value by supplying oxygen. Under such conditions the action potential also falls. In nitrogen there is little indication, when the injury potential has been diminished by stimulation, of any return to its pre-stimulation value.

We may conclude therefore that the injury potential and the action potential are the same thing looked at from different points of view, and that the injury potential represents a boundary potential in the surface of the nerve fibre maintained by some oxidative process.

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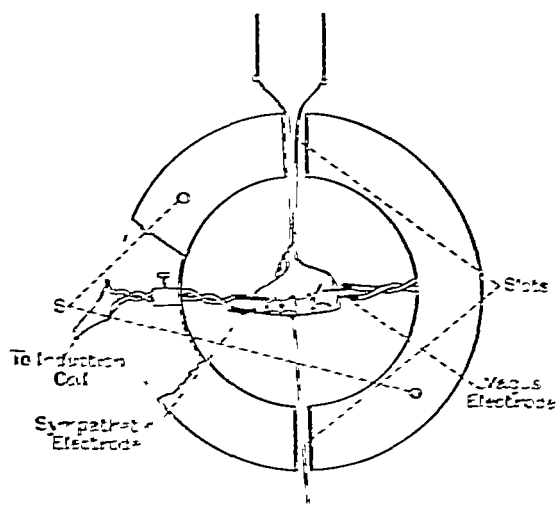
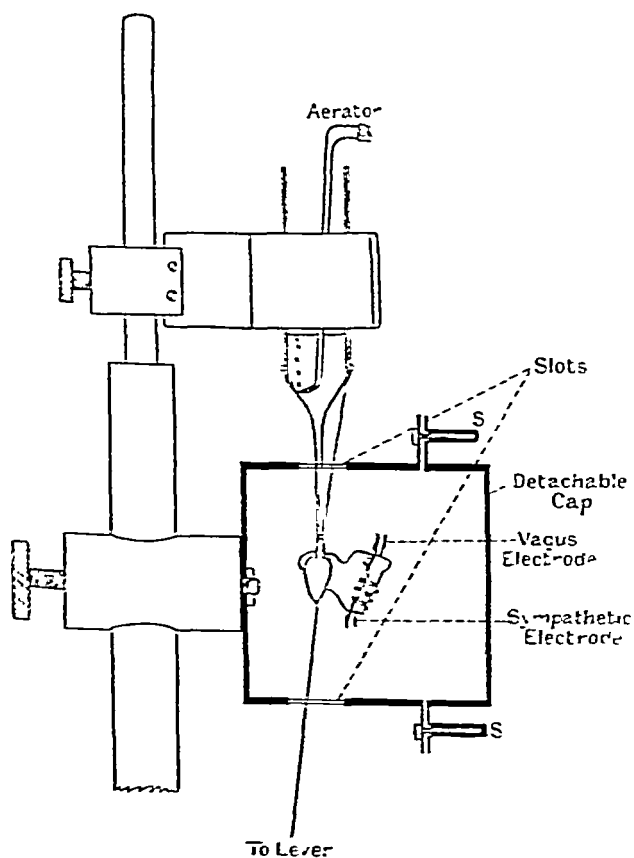
**Preparation for the measurement of the excitability of the cardiac nerves of the frog.** By B. FINKLEMAN (Manchester).

The preparation is a modification of the Loewi isolated heart-vagus preparation of the frog. It is designed to give separable vagus and sympathetic effects. A decerebrated frog is pinned out and a cut is made down the left side of its spinal column, which is then cut across at the level of the fourth spinal vertebra. The heart is next exposed, the sternum and both fore limbs being removed. The right aorta and the sinus are then ligatured and a fine-pointed glass cannula containing Ringer's solution is passed down the left aorta into the ventricle; the aorta is then tied to the cannula. The heart is now dissected out, together with a strip of tissue containing the right vago-sympathetic trunk, attached to which is the previously freed upper part of the spinal column.

The heart is now transferred to the damp chamber as in the figure, and the attached portion of spinal column is mounted on the supporting electrodes. One pair of electrodes, which are made of stout D.C.C. copper wire with platinum ends, is placed at the level of the third spinal root, one electrode being inside the spinal cord and the other resting on the outside of the vertebræ. On stimulation with a weak induction current pure sympathetic effects are obtained.

The other pair of electrodes is placed at the level of the medulla, one electrode being within the spinal canal and the other resting on the levator anguli scapulæ muscle. Stimulation of this end gives pure vagus effects if the current is weak enough to prevent spread.

To secure the survival of the nerves it has been found desirable to lead a stream of pure oxygen through the damp chamber, the atmosphere of which is kept saturated with water vapour by moist blotting paper fixed to the walls. The 1 c.c. of Ringer in the cannula is kept well stirred up by bubbling air through it. Under these conditions the heart will maintain a steady beat for many hours, the factor governing the useful life of the preparation being the time of survival of the nerves. This varies from a half to four hours. Frequently the effects of vagus and sympathetic stimulation are not lost simultaneously.



**The effect of morphia on the adrenaline content of the suprarenal glands.** By G. P. CROWDEN and M. G. PEARSON.

Owing to the lack of uniformity in the reaction to morphia in cats a series of experiments was conducted in which the conditions under which the cats were kept were very carefully controlled, particularly in regard to temperature and quiet.

The following table shows that following an injection of 20 mg. of morphia there is no depletion of the innervated gland in relation to the denervated gland provided the animal is quiet, undisturbed and kept warm, whereas if the animal is loose in the laboratory in strange surroundings and thereby excited, considerable depletion of the innervated gland occurs.

Furthermore, if the animal is kept in cold air subsequent to the injection of morphia and is not disturbed, then again a depletion of the innervated gland occurs.

It would appear therefore that morphia alone does not cause exhaustion of the adrenaline content of the gland, but it certainly renders the animal more excitable, morphia + excitement causing a definite depletion of the gland. Again, morphia without excitement but with cold, causing a definite drop in body temperature, brings about a depletion of the innervated gland. The experiments were performed on male cats.

Exp.	Operative procedure	Conditions	Relative content of glands		Fall in body temperature (rectal)
			Denervated	Innervated	
1.	Left splanchnic nerves and nerves to left semilunar ganglion cut. 9 days	Injection 20 mg. morphia. Cage 8 hr. Quiet	100	96	—
2.	Ditto. 7 days	20 mg. morphia. Cage 8 hr. Quiet	100	104	—
3.	Ditto. 14 days	20 mg. morphia. Loose in laboratory for 7 hr. Excited	100	66.5	—
4.	Ditto. 7 days	30 mg. morphia. Loose in laboratory for 5 hr. Excited	100	68.5	—
5.	Ditto. 18 days	20 mg. morphia. Cold air. Cage 3 hr. Quiet	100	74.5	38–32° C. =6° C.
6.	Left nerves severed by ligature immediately prior to injection	30 mg. morphia. Cold air. Cage 3 hr. Quiet	100	54	38–34° C. =4° C.

The expenses of this research have been defrayed by grants from the British Medical Association and the Medical Research Council.

**The biological action of iodides.** By JOHN FREUD.

In order to ascertain whether the action of thyroxin might be due to its slow conversion into iodides, the effect on the basal metabolic rate of minute amounts of iodine ion was studied. Slow intravenous injections of a 0.0006 p.c. solution of KI in Ringer's solution were made into chloralosed dogs in which the gaseous metabolism was measured. The amounts of iodide found to be efficient was  $40\gamma$  ( $1/25$  mg.), injected during half an hour, at the end of which time the metabolism of gases was once more measured. The determinations of the gaseous metabolism were carried out in three different ways. In a preliminary series<sup>1</sup> the tracheal cannula was temporarily connected (5–10 min.) with a large glass vessel of some 13 litres and the change of the composition of air in it was determined (Table I, Exps. 1–6, and *a–c*).

In the second series the inspired air was measured and samples of the expired air taken from a 10-litre Douglas bag were analysed (Table I, Exps. I–VIII, and *A–B*).

In the third series (Table II) the  $O_2$  consumption was graphically registered by means of a Krogh's spirometer.

The three methods gave similar results, which are comparable with certain reservations.  $O_2$  measure is the more reliable index of the change. The R.Q. is variable. In 20 out of 22 experiments the dogs reacted to the injection of KI into the jugular vein by an increase of the respiratory exchange within half an hour from the beginning of injection. Less or no response occurred to saphenous injection. The body temperature was carefully observed and kept as constant as possible; even when it fell slightly the increase of metabolism was still observed. In several cases trembling occurred. Of 8 thyroidectomised dogs 3 reacted to large doses.

Injection into the left jugular vein gives an average increase of  $O_2$  consumption of 31.5 p.c. (Exps. 1–6) and 30.2 p.c. (Exps. I–VIII). In Exps. VI–VIII an increase of 52.6 p.c. occurs when KI is used, while equivalent quantities of Ringer's fluid alone have no appreciable effect. Injection into the saphenous vein on the day before or the day after the jugular injection, causes in Exps. 3–6 an average decrease of 3.7 p.c., in II–VIII (small dogs) an average increase of only 15.5 p.c. Ringer's fluid in V, VII, VIII gave a decrease of 1.74 p.c., *i.e.* negligible change. In Table II, when the jugular was used (5 dogs), the average increase was 40.1 p.c. and when the saphenous was used (as in  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\zeta$ ) only 3 p.c. Injection of KI into the jugular immediately after the saphenous

<sup>1</sup> Done at the Collège de France with collaboration of E. Czarnecki.

injection gave an increase of 37.4 p.c. ( $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\zeta$ ), thus showing the importance of the way of administration. Thyroidectomised dogs *a*, *c*, *A*, *B*, show a decreasing or stable tendency (Table I) and *b* with a comparatively large dose an increase of 14 p.c. In Table II  $c_1$  did not react, to 40 $\gamma$   $b_1$  reacted with 3.5 p.c. increase, to 80 $\gamma$   $a_1$  with 13 p.c. and  $b_1$  with a slow increase from 14–31.5 p.c.

Table I.

*Oxygen consumption in c.c. per minute and kilo.*

Exp.	Dog	Injection into						Remarks
		Jugular			Saphenous			
		Before injection	Of Ringer	Of KI	Before injection	Of Ringer	Of KI	
I	22.3 K	3.9	—	5.7	—	—	—	—
2	13	6.5	—	9.6	—	—	—	—
3	17 "	4.7	—	6.9	5.1	—	3.6	—
4	20 "	5.6	—	6.5	4.9	—	4.9	—
5	10.5 "	6.8	—	7.7	7.5	—	8.5	NaI used
6	19.9 "	5	—	6.2	5	—	4.6	—
I	9 "	6.7	—	5.1	—	—	—	—
II	10 "	5.8	—	6.4	5.4	—	5.2	—
IV	7.5 "	6.7	—	7.5	7.4	—	7.5	—
V	7.5 "	—	—	—	7.2	7.4	8	—
VI	7.5 "	10.3	9.1	18.2	8.3	—	11.6	—
VII	7.5 "	8.1	9.5	12.3	9.9	10.3	13	—
VIII	6.5 "	9.7	9.3	12.1	11.3	11.9	10.1	—
a	15.3 "	6.7	—	6	—	—	—	65 $\gamma$
b	12.7 "	7.2	—	8.2	—	—	—	70 $\gamma$
c	25.2 "	4.6	—	4.4	—	—	—	79 $\gamma$
A	9 "	14.1	—	12.1	—	—	—	Tetany
B	10.8 "	5.8	—	5.9	—	—	—	—

Table II.

*Percentage difference in O<sub>2</sub> consumption as found with Krogh's Spirometer.*

Exp.	Dog	Injection into						Jugular after saphenous
		Jugular			Saphenous			
		Of Ringer	1st KI	2nd KI	Of Ringer	KI		
<i>a</i>	♀ 15.5 K	0	28	—	—	—	—	
<i>β</i>	♀ 8.5 "	0	62	—	0	0	20	
<i>γ</i>	♀ 9.2 "	-6.6	6.6	33.3	—	4.3	17.4	
<i>δ</i>	♀ 12 "	—	37.5	—	—	7.6	41.6	
<i>ε</i>	♀ 11.5 "	—	66.6	—	—	—	—	
<i>ζ</i>	♀ 14.2 "	—	—	—	—	-2.9	70.6	
<i>η</i>	♀ 9.5 "	—	—	—	—	-2.2	-27.2	
<i>a</i> <sub>1</sub>	♀ 9.5 "	—	13	—	—	—	80 γ	
<i>b</i> <sub>1</sub>	♀ 29.5 "	—	3.5	14	—	—	15 min. later 31.5	
<i>c</i> <sub>1</sub>	♂ 16 "	—	0	0	—	—	2nd injection 80 γ	

# A smooth muscle vagus nerve preparation.

By M. RABINOVICH.

Experiments were made with smooth muscle nerve preparations, the smooth muscle strip being taken from the fundus and body of the stomach or the œsophagus with the vagus nerve attached. Under ether anaesthesia the stomach and œsophagus were removed. The vagus was identified at the upper part of the œsophagus and dissected down. The strip of muscle to which the nerve was traced was separated from the mucous membrane and its ends fixed by means of Michel clips. The preparation was left in Tyrode solution at  $37^{\circ}$  C. for half an hour and then placed in a muscle chamber as previously described (1). The chamber was filled with Tyrode solution maintained at  $37^{\circ}$  C. and at pH 7.5 by means of a thermostat and a constant current of oxygen and carbon dioxide bubbled through it. The muscle was attached to an isometric lever fitted with a small galvanometer mirror and optical records were taken on stimulation of the

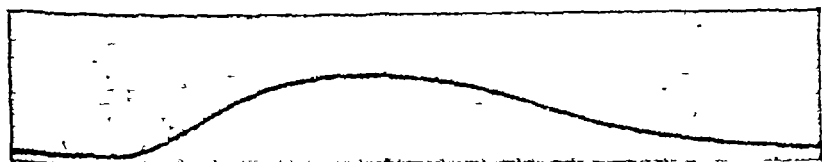


Fig. 1. Response of circular strip of smooth muscle of cat's œsophagus to single induction shock. Time intervals = 0.2 sec.  $\Delta$  = point of stimulation.

nerve fibre. A time marker recorded 0.2 sec. intervals and a signal marker was in series with the stimulating current. The nerve was stimulated by means of platinum electrodes kept outside the solution.

The make or break of a constant current has so far not proved capable of causing stimulation. A single induction shock causes a contraction in a minority of cases. Faradic stimulation causes a contraction in practically all the preparations made. The latent periods are given in Table I. They are fairly constant for a given preparation but vary over a fairly wide range for different strips of muscle. The shortest interval recorded was 0.38 sec.; the longest latent periods were observed when only a small response was obtained. With a single induction shock a smooth curve is obtained; contraction lasts 1.5–2.5 sec. and is fairly constant for a given strip. Relaxation is slower and takes 5 or more seconds to complete, though the first part is more rapid. Half relaxation occurs within 1.5 sec. Faradic stimulation of a circular preparation gives

a response similar to that obtained with a single shock. The contraction lasts 1.5-3 sec. Relaxation is again much slower. Continuation of the stimulation appears to delay the relaxation. The longitudinal muscle gives a more sustained contraction with faradic stimulation; the contraction seems to consist of a series of waves superimposed upon one another, lasting for 30 sec. or more when relaxation slowly occurs.

A preparation is very quickly fatigued and even after a short period of stimulation it is necessary to allow a few minutes rest, otherwise a smaller response with a longer latent period is obtained. Repeated stimulation rapidly abolishes the response.

Preparations of the striped muscle of the upper end of the cat's œsophagus with the recurrent laryngeal nerve give a typical striped muscle response. Strips from intermediate regions give a mixed response in which the striped and unstriped elements can be clearly discriminated.

Table I

Preparation	Latent period sec.	Number of observations	Type of stimulation
Circular œsophagus	Average: 0.55 Longest: 0.75 Shortest: 0.38	33	Faradic
Circular œsophagus	Average: 0.52 Longest: 0.7 Shortest: 0.4	8	Single induction shock
Longitudinal œsophagus	Average: 0.47 Longest: 0.39 Shortest: 0.52	7	Faradic
Stomach	Average: 0.7 Longest: 0.65 Shortest: 0.75	6	Faradio

1. McSwiney and Newton. *This Journ.* 63, p. 51. 1927.

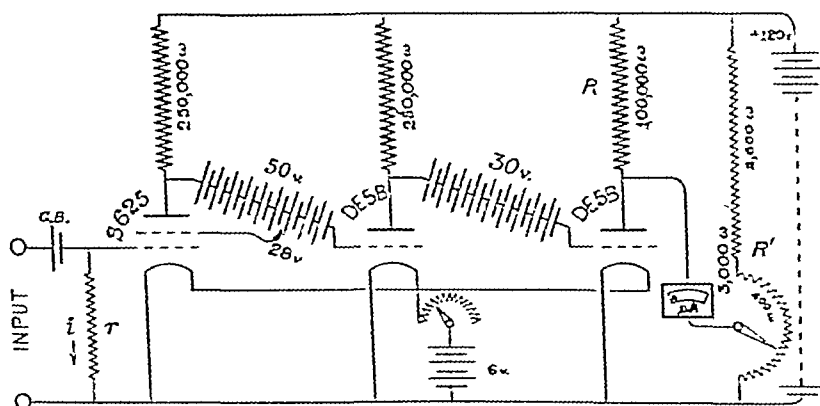
• **Three-stage amplifier for bioelectric currents and potential differences.** By D. T. HARRIS.

This assembly was demonstrated at the annual meeting of the Society when the variations in P.D. of an amphibian heart were shown on a portable galvanometer giving full scale deflexion when used as a micro-ammeter.

The general design of the amplifier follows standard practice<sup>(1)</sup> and the magnitudes of the various electrical quantities have been estimated with a view to stability and ease of working. When applied to biological tissues no screening or earthing will be found necessary on account of the heavy damping thus introduced into the grid circuit of the first valve;

wire-wound anode resistances with low distributed capacity should be chosen and accumulators, in virtue of their low internal resistance and their steady E.M.F., are advisable—the large WH (Exide) type for the main H.T. battery and the small WJ for the coupling batteries. Under these circumstances oscillation will be avoided and the zero will be found remarkably steady—no change being seen on a C.S.I. pointer galvanometer reading 1 division per  $\frac{1}{2}$  micro-amp. The amplifier has not been tested on a more sensitive instrument since its sole object is to render the minute electrical changes of living tissues visible on a handy type of robust recording instrument.

When used for an A.C. input the mode of working is apparent. On the application of a direct current  $i$  across the grid leak  $r$  (2 megohms is



a suitable value) there is a voltage drop at the control grid of magnitude  $ir$  which alters the plate current of the first valve and a similar enhanced change occurs in the subsequent stages, part of the change in plate current of the last valve being indicated by the micro-ammeter  $\mu A$ . By making  $R$  large compared with  $R'$  the bulk of the plate current change is diverted through the galvanometer.

If a high magnification is required, the more usual DE5B valve (Marconi) in the first stage may be replaced, as shown in the figure, by an S625 valve<sup>(2)</sup>, the screen being led off to the + 28 v. tapping on the coupling battery; if still more magnification is required, a similar replacement may be effected in the second stage also, or another method may be tried, namely, that of giving the first valve an independent large H.T. battery and coupling its anode resistance at the anode end only, by means of the coupling battery; by this latter method the valve can be made to operate under its optimum conditions.



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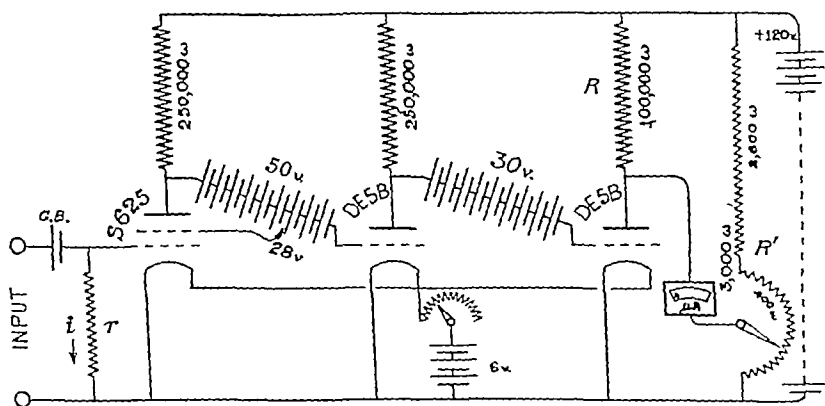
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The inclusion of a potentiometer in the grid circuit of the first valve is a useful means of setting the grid bias G.B. (usually about 0 to  $-\frac{1}{2}$  volt) which is rather critical; the permissible range is not a large one, and this is not essential since only small variations can be applied to so powerful an amplifier. The potentiometer serves the further useful purpose of a quick and ready means of calibrating the amplifier. It is advisable, as with other amplifiers, to keep the grid leads as short as possible and to place the valve with the largest emission in the last stage. The inclusion of a micro-ammeter in the grid lead solves any difficulty in adjusting the voltage of the coupling battery.

For D.C. inputs and A.C. of frequencies up to half a million per sec. this amplifier is practically distortionless; for extremely high frequencies the resistances and valve inter-electrode capacities will constitute sources of distortion.

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Adrian, E. D. *This Journ.* 62, p. 49. 1926. *Ergeb. d. Physiol.* 27. p. 501. 1928.
2. Round, H. J. *The Shielded Four-electrode Valve.* (Cassell & Co., 1927.)

